(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 March 2003 (06.03.2003)

PCT

(10) International Publication Number
WO 03/018053 A1

(51) International Patent Classification[7]: A61K 39/04, C07K 14/35, A61P 31/06

(21) International Application Number: PCT/EP02/09345

(22) International Filing Date: 21 August 2002 (21.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01120194.4 22 August 2001 (22.08.2001) EP

(71) Applicant *(for all designated States except US)*: **MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.** [DE/DE]; Berlin (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GRODE, Leander** [DE/DE]; Dönhoffstr. 32, 10318 Berlin (DE). **JUNG-BLUT, Peter, R.** [AT/DE]; Reichensteiner Weg 9, 14195 Berlin (DE). **KAUFMANN, Stefan, H., E.** [DE/DE]; Am Rosenanger 57a, 13465 Berlin (DE). **MATTOW, Jens** [DE/DE]; Kühlebornweg 17, 12167 Berlin (DE). **MOLLENKOPF, Hans-Joachim** [DE/DE]; Berchtesgadener Str. 5, 10779 Berlin (DE). **SCHAIBLE, Ulrich** [DE/DE]; Sensburger Allee 22a, 14055 Berlin (DE).

(74) Agent: **VOSSIUS & PARTNER**; Siebertstrasse 4, 81675 München (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/018053 A1

(54) Title: VACCINE AGAINST MYCOBACTERIAL-INDUCED DISEASES

(57) Abstract: The present invention relates to a pharmaceutical composition comprising at least one polypeptide selected from the group consisting of (a) a polypeptide encoded by a polynucleotide comprising a nucleic acid sequence as defined herein; (b) a polypeptide comprising an amino acid sequence as defined herein; (c) a polypeptide which is or which comprises a functional domain, an antigenic fragment and/or a fragment capable of eliciting and/or triggering an immune response in a mammal of the polypeptide of(a) or (b); (d) a polypeptide which is encoded by a polynucleotide which is at least 80 % identical to the polynucleotide as defined in (a) and which is capable of eliciting and/or triggering an immune response in a mammal; and (e) a polypeptide which is encoded by a polynucleotide which hybridizes under stringent conditions with the polynucleotide as defined in (a) or (d) and is capable of eliciting and/or triggering an immune response in a mammal. Furthermore, the invention provides for pharmaceutical compositions comprising fusion proteins, polynucleotides, vector(s), host cell(s) or antibodies as described herein. In addition, the invention relates to recombinant (bacterial) host cells and methods for the production of a vaccine.

Vaccine against mycobacterial-induced diseases

The present invention relates to a pharmaceutical composition comprising at least one polypeptide selected from the group consisting of (a) a polypeptide encoded by a polynucleotide comprising a nucleic acid sequence as defined herein; (b) a polypeptide comprising an amino acid sequence as defined herein; (c) a polypeptide which is or which comprises a functional domain, an antigenic fragment and/or a fragment capable of eliciting and/or triggering an immune response in a mammal of the polypeptide of (a) or (b); (d) a polypeptide which is encoded by a polynucleotide which is at least 80% identical to the polynucleotide as defined in (a) and which is capable of eliciting and/or triggering an immune response in a mammal; and (e) a polypeptide which is encoded by a polynucleotide which hybridizes under stringent conditions with the polynucleotide as defined in (a) or (d) and is capable of eliciting and/or triggering an immune response in a mammal. Furthermore, the invention provides for pharmaceutical compositions comprising fusion proteins, polynucleotides, vector(s), host cell(s) or antibodies as described herein. In addition, the invention relates to recombinant (bacterial) host cells and methods for the production of a vaccine.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated by reference.

Tuberculosis (TB), caused by mycobacteria of the *tuberculosis* complex, mostly *M. tuberculosis*, is a major infectious disease. According to the World Health Organization each year about 8 million new cases of TB are notified globaly, 2 million of which prove fatal [1]. The currently available vaccine against TB, *Mycobacterium bovis* Bacillus Calmette-Guérin, was originally developed by Albert Calmette and Camille Guérin. It is a viable vaccine that was attenuated by 230 passages in vitro between 1908 and 1921 [2-3]. Today several distinct substrains of

M. bovis BCG with different degrees of gene deletions exist [4]. The necessity to develop a novel vaccine against TB has been recognized for many years. It results from the observation, that BCG prevents miliary and meningeal TB in children, but rather fails to protect adults effectively against pulmonary TB, the most prevalent form of the disease [5-6].

In 1998 the entire DNA sequence of *M. tuberculosis* strain H37Rv with a total of 3924 ORFs (according to the gene classification of the Sanger Center) was published [7]. In the meantime, the genomes of 2 other mycobacterial strains, *M. tuberculosis* clinical isolate CDC1551 and *Mycobacterium leprae* strain TN, have been completely sequenced and the sequencing projects of 4 additional mycobacterial strains including *M. bovis* strain AF2122/97 are nearing completion [8-10]. At the DNA level *M. tuberculosis* shares > 99.9% identity with wild-type *M. bovis* and the various BCG substrains. Although highly related, *M. tuberculosis* and *M. bovis* can be distinguished on the basis of their host range, virulence and physiological properties [11]. To date our knowledge of the genetic basis for these phenotypic differences, as well as for the virulence of *M. tuberculosis* and for the attenuation of BCG is still fragmentary. Comparative genomic hybridization experiments have revealed that *M. bovis* and the different BCG substrains lack distinct regions of the genome of *M. tuberculosis* H37Rv [4, 12-16]. For example, the BCG substrains Chicago and Copenhagen examined in this study lack 13 regions of the genome of *M. tuberculosis* H37Rv comprising 111 ORFs.

A complementary approach towards comparative analysis of *M. tuberculosis and M. bovis* BCG strains employs proteomics, e.g. protein separation by 2-DE (or liquid chromatography) in combination with protein identification and characterization by MS and other protein analytical methods. 2-DE allowed us to separate mycobacterial whole cell preparations into approximately 1 800 distinct protein species. A protein species is defined by its chemical structure [17]. One has to be aware that within a 2-DE gel proteins with differential electrophoretic mobility do not necessarily have to represent proteins encoded by different ORFs. They may also be identified as different protein species of the same protein, reflecting a

diversification of a primary translation product by co- and post-translational protein modifications.

The analysis of mycobacterial proteins from 2-DE gels by MS is facilitated by the availability of the entire DNA sequence of *M. tuberculosis* H37Rv. 2-DE has been applied to analysis of mycobacteria in several laboratories [12, 18-28].

Inhibiting the spread of TB will require effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient method for inducing protective immunity. The most common Mycobacterium for this purpose is Bacillus Calmette-Guerin (BCG), an avirulent strain of *Mycobacterium bovis*.

However, the safety and efficacy of BCG is a source of controversy, and some countries, such as the United States and Belgium, do not vaccinate the general public.

Additionally, it has been shown that BCG vaccination affords greater protection against leprosy than against tuberculosis (Ponninghaus, Lancet 339 (1992), 639).

Furthermore, *M. bovis* BCG has failed to protect against mycobacterial induced diseases, in particular TB, in several trials (WHO, Tech. Rep. Ser. (1980), 651, 1-15) for reasons that are not entirely clear (Fine, Tubercle 65 (1984), 137-153).

Additionally, it has been shown that the vaccine strain of M. bovis BCG only confers protection against the severe form of miliary tuberculosis in children (Fine, Lancet 346 (1995), 1339-1345). In contrast, its protective capacity against the most common form, pulmonary tuberculosis in adults, is low and highly variable (Colditz (1994), JAMA 271, 698).

Diagnosis of TB is commonly achieved using a skin test, which involves intradermal exposure to tuberculin PPD (protein-purified derivative). Antigen-specific T cell responses result in measurable induration at the injection site by 48-72 hours after injection, which indicates exposure to Mycobacterial antigens. Sensitivity and specificity have, however, been a problem with this test, and individuals vaccinated with BCG cannot be distinguished from infected individuals.

Therefore, it is of major concern that effective and safe vaccines and therapies for the immunization and the treatment of mycobacterial-induced diseases/disorders.

The technical problem of the present invention was thus to provide compositions useful for effective immunization against pathogenic organisms, in particular mycobacteria, for effective therapy of infected humans and animals that can be reliably used in low doses and with substantially no side effects.

The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a pharmaceutical composition comprising at least one polypeptide selected from the group consisting of

(a) a polypeptide encoded by a polynucleotide comprising a nucleic acid sequence as shown in SEQ ID NO: 1;

(b) a polypeptide comprising an amino acid sequence as shown in SEQ ID NO: 2;

(c) a polypeptide which is or which comprises a functional domain, an antigenic fragment and/or a fragment capable of eliciting and/or triggering an immune response in a mammal of the polypeptide of (a) or (b);

(d) a polypeptide which is encoded by a polynucleotide which is at least 80% identical to the polynucleotide as defined in (a) and which is capable of eliciting and/or triggering an immune response in a mammal; and

(e) a polypeptide which is encoded by a polynucleotide which hybridizes under stringent conditions with the polynucleotide as defined in (a) or (d) and is capable of eliciting and/or triggering an immune response in a mammal.

In the present invention, it was surprisingly found that a gene product encoded by Rv1511, a putative GDP-D-mannose dehydratase, is a potent vaccination candidate against diseases caused or elicidated by bacterial strains, in particular virulent Mycobacteria (e.g. M. tuberculosis). Said gene product is not limited to full length gene products but also to fragments thereof as descibed herein below.

Proteins present in virulent *M. tuberculosis* but absent in the attenuated *M. bovis* BCG vaccine represent putative virulence factors. It has been proposed that these gene products are also interesting antigen candidates for rational vaccine design against TB. *M. bovis* is the causative agent of cattle TB, and in rare cases it can also cause human TB. Therefore, those proteins of *M. tuberculosis* which are encoded by genes that are missing in *M. bovis* BCG but not in wild-type *M. bovis* (e.g. proteins encoded by ORFs from the deletion regions RD1-2; RD8; RD14; and RD16 of the genome of *M. tuberculosis* H37Rv) are of particular interest. The best known of these are: the early secretory antigen target (ESAT-6; encoded by Rv3875 from RD1) and the secreted immunogenic protein Mpb64/Mpt64 (MPT64; encoded by Rv1980c from RD2). Both are secreted proteins of unknown function, which have been described as T cell antigens, with ESAT-6 being more effective than MPT64 in stimulating interferon γ production in murine and human T cells and in inducing partial protection to TB in mice [54 to 57]. In contrast, *M. tuberculosis* specific proteins encoded by genes deleted in both *M. bovis* BCG and wild-type *M. bovis* (e.g. proteins encoded by ORFs from the deletion regions RD3-RD7; RD9-13; and RD15 of the genome of *M. tuberculosis* H37Rv) were proposed to be unlikely to be good vaccine candidates. Therefore, in this invention it was surprisingly found that putative GDP-D-mannose dehydratase encoded by Rv1511 located in the deletion region RD6 of the genome of *M. tuberculosis* H37Rv (a region that is missing in both virulent wild-type *M. bovis* and attenuated *M. bovis* BCG) may provide constant protection against TB-challenge over all time points examined. Furthermore, it is of note that encoded RV1511 and its fragments have highly homologous counterparts in mammalian species, like, inter alia, in mouse and human. Accordingly, it is surprising that a putative, bacterial GDP-D-mannose dehydratase (and fragments thereof) is capable of eliciting and/or triggering an immune response in mammals.

In context with this invention the term "Rv1511" relates to a deduced open reading frame as described in Cole (1998), Nature 393, 537. Rv1511 (RD6) was further described by Behr, Science 284 (1999), 1520 and described as differentially expressed in M. tuberculosis and M. bovis in WO 00/44392. Yet, Rv1511 had never been proposed as a potential vaccine candidate since said (expressed) ORF appears to be missing in virulent and attenuated M. bovis strains.

The term "polypeptide" means, in accordance with the present invention, (a) peptide(s) or (a) (poly)peptide(s) which encompass amino acid chains of any length, wherein the amino acid residues are linked by covalent peptide bonds. However, peptidomimetics of such proteins wherein amino acid(s) and/or peptide bond(s) have been replaced by functional analogs are also encompassed by the invention. In accordance with this invention, a protein may comprise different protein species. A protein species is (furthermore and not limiting) defined by its chemical composition and modifications of said peptide(s)/(poly)peptide(s) by, inter alia, glycosylations, acetylations, phosphorylations, lipidations or by amino acid exchanges, the term describes a chemically clearly-defined molecule and corresponds, inter alia, to one spot on a high-performance 2-DE pattern (Jungblut, Electorphoresis 17 (1996), 839-847). The term protein species is therefore defined as the smallest unit of a protein classification, defined by its chemical structure.

Most preferably, the polypeptide encoded by a nucleic acid sequence as shown in SEQ ID NO: 1 is to be employed in the pharmaceutical composition of this invention. Said polypeptide has preferably the amino acid sequence as shown in SEQ ID NO: 2. Yet, in accordance with this invention homologous polypeptides may be employed which are capable of elicidating an immunobiological response in a mammal, preferably in a human. Preferably said polypeptides are at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90%, more preferably at least 95%, and most preferably at least 99% identical to the polypeptide as shown in SEQ ID NO: 2.

The polypeptide to be employed in the pharmaceutical composition of this invention preferably is a putative GDP-D-mannose-dehydratase, preferably of M. tuberculosis, more preferably a putative GDP-D-mannose-dehydratase as deposited with NCBI as B70714 or CAB02025. Corresponding polynucleotides/nucleic acid molecules, encoding for the Rv1511 gene product can be found in NCBI under the accession number Z79701 (*M. tuberculosis* H37Rv complete genome; segment 65/162; strand: plus, nucleotides: 30672-316949) and AD000001 (*M. tuberculosis* sequence from clone y456; strand: minus; nucleotides: 10295-9273).

Preferably, the polynucleotide encoding for Rv1511 is at least 80%, more preferably at least 90%, and most preferably at least 95% homologous to the nucleic acid sequence as shown in SEQ ID NO: 1.

The term "composition", as used in accordance with the present invention, comprises at least one protein, an antigenic fragment of said protein, a fusion protein, a nucleic acid molecule and/or an antibody as defined herein and, optionally, further molecules, either alone or in combination, like e.g. molecules which are capable of optimizing antigen processing, cytokines, immunoglobulins, lymphokines or CpG-containing DNA stretches. The composition in form of a vaccine may include an adjuvant. Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminium slats (alum), such as aluminium hydroxide, aluminium phosphate, aluminium sulfate, etc.; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example, (a) MF59™ (WO 90/14837; Chapter 10 in Vaccine design: the subunit and adjuvant approach, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing MTP-PE) formulated into submicron particles using a microfluidizer, (b) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as QS21 or Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS may be devoid of additional detergent, e.g. WO 00/07621; (4) Complete Freund's Adjuvant (CFA) in Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (e.g. IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (WO 99/44636), etc.), interferons (e.g. gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.;

(6) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) e.g. GB-2220221, EP-A-0 689 545, optionally in the substantial absence of alum when used with pneumococcal saccharides e.g. WO 00/56358; (7) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions e.g. EP-A-0 835 318, EP-A-0 735 898, EP-A-0 761 231; (8) oligonucleotides comprising CpG motifs (Roma, Nat. Med. 3 (1997), 849-854; Weiner, PNAS USA 94 (1997), 10833-10837; Davis, J. Immunol. 160 (1998), 870-876; Chu, J. Exp. Med. 186 (1997), 1623-1631; Lipford, Eur. J. Immunol. 27 (1997), 2340-2344; Moldoveanu, Vaccine 16 (1988), 1216-1224; Krieg, Nature 374 (1995), 546-549; Klinman, PNAS USA 93 (1996), 2879-2883; Ballas, J. Immunol. 157 (1996), 1840-1845; Cowdery, J. Immunol. 156 (1996), 4570-4575; Halpern, Cell Immunol. 167 (1996), 72-78; Yamamoto, Jpn. J. Cancer Res. 79 (1988), 866-873; Stacey, J. Immunol. 157 (1996), 2116-2122; Messina, J. Immunol. 147 (1991), 1759-1764; Yi, J. Immunol. 157 (1996), 4918-4925; Yi, J. Immunol. 157 (1996), 5394-5402; Yi, J. Immunol. 160 81998), 4755-4761; and Yi, J. Immunol. 160 (1998), 5898-5906; International patent applications WO 96/02555, WO 98/16247, WO 98/18810, WO 98/40100, WO 98/55495, WO 98/37919 and WO 98/52581) i.e. containing at least one CG dinucleotide, with 5-methylcytasine optionally being used in place of cytosine; (8) a polyoxyethylene ether or a polyoxyethylene ester e.g. WO 99/52549; (9) a polyoxytheylene sorbitan ester surfactant in combination with an oxtoxynol (WO 00/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (WO 00/21152); (10) a saponin and an immunostimulatory oligonucleotide (e.g. a CpG oligonucleotide) (WO 00/62800); (11) an immunostimulant and a particle of metal salt e.g. WO 00/23105; (12) a saponin and an oil-in-water emulsion e.g. WO 99/11241; (13) a saponin (e.g. QS21) + 3dMPL + IL-12 (optionally + a sterol) e.g. WO 98/57659; (14) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum (especially aluminium phosphate and/or hydroxide) and MF59 are preferred for use with the saccharide antigens of the present invention.

The composition may be in solid, liquid or gaseous form and may be, inter alia, in form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). In a preferred

embodiment, said composition comprises one protein (Rv1511) combined with at least one, preferably two, preferably three, more preferably four, most preferably five differentially expressed proteins. Differentially expressed proteins, in particular of mycobacteria are known in the art and, inter alia, described in WO 00/44392.

The polypeptides/proteins to be employed in the pharmaceutical composition of the invention may be produced by, for example, recombinant techniques or by biochemical or synthetic techniques which are known to the skilled artisan (Sambrook et al., "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Laboratory, N.Y. (1989); Ausubel, "Current Protocols in Molecular Biology", Green Publishing Associates and Wiley Interscience, N.Y. (1989)).
The differential protein expression of the gene product of Rv1511, as defined herein, can be detected by preparation of microorganisms or, less preferred, compartments/fragments thereof, 2-DE, subtractive analysis and identification of proteins by peptide mass fingerprinting (PMF) with or without confirmation by further methods.

The term "virulent strain", in accordance with the present invention, denotes the capacity of a pathogenic strain of Mycobacteria to infect a host and/or to cause disease - defined broadly in terms of severity of symptoms in a host. Thus, a "virulent strain" might cause symptoms in a susceptible host, whereas another host might be unaffected by this strain, which can be therefore considered as being an "avirulent strain" in this second host. As used in accordance with the present invention, the term "avirulent strain" denotes strains of a Mycobacteria which is not capable of inducing infection and/or causing disease in a specific host or in a host species. The term "avirulent strains" denotes furthermore attenuated strains of microorganisms.

The term "antigenic fragment", as used herein, refers to the ability of said fragment to elicit an immune response (e.g. humoral or cellular) in a subject, such as a human, and/or in a biological sample. These fragments may consist entirely of the antigenic and/or immunogenic portion of the protein or may contain additional sequences. The additional sequences may be derived from said protein or may be

heterologous, and such additional sequences may (but need not) be antigenic and/or immunogenic. As shown herein and in the appended examples, antigenic peptides/fragments of expressed Rv1511 may be deduced by computer-assisted methods. Preferred "antigenic fragments" are shown, but not limited to, the peptide-stretches as shown in Table 1 of appended example 3.

Antigenic fragments may be produced recombinantly using a polynucleotide sequence that encodes the antigenic fragment or may be produced by biochemical or synthetic techniques. Those methods are known to those of ordinary skill in the art (see, e.g. Sambrook et al., loc. cit.; Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, NY (1988); Merrifield, J. Am. Chem. Soc. 85 (1963), 2149-2146; Stewart, "Solid Phase Peptide Synthesis", WH Freeman Co, San Francisco (1969); Scopes, "Protein Purification", Springer Verlag, New York, Heidelberg, Berlin (1987); Janson, "Protein Purification, Principles, High Resolution Methods and Applications", VCH Publishers, New York, Weinheim, Cambridge (1989); Wrede, "Concepts in Protein Engineering and Design", Walter de Gruyter, Berlin, New York (1994); Wittmann-Liebold, Jungblut "Analysis and Characterization of Proteins", 47-107). Accordingly, and in context of the present invention, the term "antigenic fragment" also relates to the polynucleotide sequence encoding the antigenic fragment as, inter alia, depicted in Table 1 of appended example 3.

The term "fragment capable of eliciting and/or triggering an immune response in a mammal" relates, in accordance with the invention to fragments of Rv1511 as defined herein which are capable of eliciting said immunological responses in a mammal, in particular in mouse, cattle (bovine) and most preferably in humans. Preferably said fragments comprise at least 8, preferably at least 9, more preferably at least 10, more preferably at least 12, more preferably at least 14, more preferably at least 16, more preferably at least 18 and more preferably at least 20 amino acids, in particular when B-cell stimulation is desired. Should the elucidation of a CD8 T-cell response be envisaged, said fragments comprise preferably at least 8, more preferably at least 9, most preferably at least 10 amino acids. However, in B-cell as well as in CD4 T-cell stimulations, fragments of Rv1511 may be employed which are considerably longer or which are comprised in fusion proteins or peptides as described herein. As documented in the appended examples fragments of RV1511

to be employed in context of this invention may comprise the fragments as depicted in Table 1 or as shown in SEQ ID NOs: 3 to 48. Particular preferred fragments are fragments as shown in SEQ ID NOs: 13, 20, 23, 24, 29, 35 and 42. The appended examples furthermore illustrate and document that such fragments are particularly useful in the preparation of a composition, preferably of a pharmaceutical composition, for the treatment as well as for the prevention of a disease related to a mycobacterial infection.

In accordance with this invention, and as disclosed herein, it is also envisaged that polynucleotides/nucleic acid molecules encoding fragments of Rv1511 as described herein are to be employed in pharmaceutical compositions described herein. The person skilled in the art can deduce antigenic fragments and/or fragments of Rv1511 capable of eliciting and/or triggering immune responses in mammals by methods known in the art, which comprise not only computer-assisted predictions (as documented in appended examples) but also in vitro and in vivo experiments. Said in vivo experiments also comprise the use of test animals, like mice or rats. Furthermore, clinical trials are envisaged after safety and efficacy has been established in pre-clinical experiments. Further methods to be employed in order to verify antigenic fragments or the potency of fragments of Rv1511 to elicit an immune response are, but are not limited to, enumeration of antibody or cytokine producing cells by cytokine enzyme-linked ImmunoSPOT (ELISPOT) technique (Mollenkopf, Vaccine 19 (2001), 4028) or by flow cytometric cell analysis and cell sorting (FACS) (Nielsen, J. Immunol. 165 (2000), 2287; Appay, J. Exp. Med. 192 (2000), 63), antigen-specific lymphocyte proliferation (Huong, J. Immunol. Methods140 (1991), 243), MHC restricted antigen-specific cytolysis (Munk, J. Immunol. 143 (1989), 2844; Silva, Infect. Immun. 68(2000), 3269) and cell phenotyping. It is of note that the fusion protein as described herein and to be employed in accordance with this invention may also comprise (besides Rv1511-related antigens and/or fragments thereof) antigens and/or immunogenic domains which are not restricted to Mycobacterium antigens and can be selected from autoantigens, tumor antigens and pathogen antigens such as virus antigens, parasite antigens, bacterial antigens in general and immunogenic fragments thereof. Specific examples for suitable tumor antigens for broad spectrum anti-tumor therapy are human antitumor antigens such

as members of the MAGE multigene family, such as MAGE-A to MAGE-L, BAGE and GAGE and melanocyte differentiation antigens, such as MELAN-A/MART; see Valmori, J. Immunol. 168 (2002), 4231-4240, Jager, Int. J. Cancer 98 (2002), 376-388, or Chomez, Cancer Res. 61 (2001), 5544-5551. Specific examples for suitable virus antigens are human tumor virus antigens such as human papilloma virus antigens, e.g. antigens E6 and E7, influenza virus antigens, e.g. influenza virus nucleoprotein or retroviral antigens such as HIV antigens, e.g. the HIV antigens p17, p24, RT and Env. Specific examples for suitable parasite antigens are Plasmodium antigens such as liver stage antigen (LSA-1), circumsporozoite protein (CS or allelic variants cp26 or cp29), thrombospondin related amonymous protein (TRAP), sporozoite threonine and asparagine rich protein (STARP) from Plasmodium falciparum and Toxoplasma antigens such as p30 from Toxoplasma gondii. Specific examples for suitable bacterial antigens are Legionella antigens such as Major Secretary Protein (MSP) from Legionella pneumophila.

Additionally, the invention relates to a (pharmaceutical) composition comprising a polynucleotide encoding for a fusionprotein/polypeptide as defined herein or comprising a fusion protein comprising a polypeptide and/or an antigenic fragment as defined in the above.

The protein and/or the antigenic fragment to be employed herein and being (expressed) Rv1511 or a fragment thereof can comprise a further domain, said domain being linked by covalent or non-covalent bonds. Further domains are illustratively described herein. The linkage can be based on genetic fusion according to the methods known in the art (Sambrook et al., loc. cit.; Ausubel, loc. cit.) or can be performed by, e.g., chemical cross-linking as described in, e.g., WO 94/04686. The additional domain present in the fusion protein comprising the protein of the invention may be joined directly (i.e. with no intervening amino acids) or may be linked by a flexible linker, advantageously a polypeptide linker, wherein said polypeptide linker comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of said further domain and the N-terminal end of the protein or vice versa. The above described fusion protein may further comprise a cleavable linker or cleavage site, which, for example, is specifically recognized and cleaved by proteinases or chemical agents.

Cleavable linker sequences include, but are not limited to, Factor XA or enterokinase (Invitrogen, San Diego, CA, USA).
Additionally, said further domain may be of a predefined specificity or function. In this context, it is understood that the protein to be employed in the pharmaceutical composition of the invention may be further modified by conventional methods known in the art. This allows for the construction of fusion proteins comprising the protein defined herein (Rv1511) or a functional fragment/antigenic fragment thereof and other functional amino acid sequences, e.g., immunologically relevant proteins like cytokines, lymphocytes, interferones, or protein tags (GST, GFP, h-myc peptide, FLAG, HA peptide) which may be derived from heterologous proteins. Preferably, the Rv1511 or the functional fragment thereof is capable of elucidating an immunoresponse in animals or humans and/or may confer protective immunity in animals or humans.

In addition, the invention relates to a pharmaceutical composition comprising a fusion protein comprising at least two proteins as defined herein and/or (an) antigenic fragment(s) as defined herein.

In a further embodiment, the fusion protein to be employed in the present invention for the preparation of a pharmaceutical composition comprises an immunostimulatory molecule. Yet, it is also envisaged that a pharmaceutical composition comprising a polypeptide as defined herein above or a fragment thereof (i.e. Rv1511 or a fragment thereof) further comprises, optionally, an immunostimulatory molecule.

The term "immunostimulatory molecule" denotes in accordance with the present invention molecules or fragments thereof which, inter alia, activate and/or stimulate the humoral and cellular response of an immune system. They might, e.g. activate antigen-presenting cells, stimulate natural killer cells, enhance the production of antibodies directed against an antigen and/or a pathogen or induce the proliferation of cells of the immune system. These molecules are known in the art and comprise, inter alia, cytokines, lymphokines, immunoglobulins, interleukins and/or complement

factors (see, e.g. Paul, "Fundamental Immunology", Raven Press (1989); Schaible, Adv. In Immunology 71 (1999), 261-377).

In one further preferred embodiment of said fusion protein comprises a molecule capable of optimizing antigen processing or comprises a peptide/polypeptide which is capable of eliciting and/or triggering an immune response in a mammal, preferably in a human. Similarly, pharmaceutical compositions comprising RV1511 or (a) fragment(s) thereof or comprising a polynucleotide encoding the same, may also further comprise, optionally, (a) molecule(s) capable of optimizing antigen processing.

Cellular immune recognition is mediated by a special class of lymphoid cells, T-cells. These cells do not recognize whole antigens but instead they respond to degraded peptide fragments thereof which appear on the surface of the target cell bound to proteins called major histocompatibility complex (MHC) molecules (antigen processing). Essentially all nucleated cells have MHC class I molecules, whereas MHC II are restricted to immune cells with special presenting qualities. Molecules which are capable of optimizing antigen processing are known in the art and comprise, inter alia, listeriolysin, which improves MHC class I restricted immune responses (see, e.g., Hess, PNAS 95 (1998), 5299-5304). Preferably, the immunogenic domain of Rv1511 or of the peptide/polypeptide capable of eliciting and/or triggering an immune response is capable of eliciting a T-cell mediated immune response, more preferably a MHC class I-restricted CD8 T-cell response.

Peptides/polypeptides capable of eliciting and/or triggering an immune response in a mammal, preferably in a human are preferably selected from immunogenic peptides or polypeptides from M. bovis or M. tuberculosis or from immunogenic fragments thereof. Specific examples for suitable antigens are Ag85B (p30) from M. tuberculosis, Ag85B (α-antigen) from M. bovis BCG, Ag85A from M. tuberculosis and ESAT-6 from M. tuberculosis. More preferably, the immunogenic domain is derived from antigens as disclosed in WO 99/10496.

The invention also provides for pharmaceutical compositions, in particular to vaccines to be employed before and/or after an infectious mycobacterial challenge of an individual, wherein said at least one polypeptide being or comprising a functional domain, a antigenic fragment and/or a fragment capable of eliciting and/or triggering an immune response in a mammal is a polypeptide comprising an amino acid sequence selected from the group consisting of the amino acid sequences as depicted in any one of SEQ ID NOs: 3 to 48. Said fragments are fragments derived from the amino acid sequence of Rv1511 disclosed in SEQ ID NO: 2 or as encoded by the nucleic acid sequence shown in SEQ ID NO: 1. Preferably, said functional domain, said antigenic fragment and/or said fragment capable of eliciting and/or triggering an immune response in a mammal is the peptide shown in SEQ ID NO: 13, 20, 23, 24, 29, 35 or 42.

It is also envisaged (and documented in the appended examples) that nucleic acid molecules coding for any one of the amino acid sequences as depicted in any one of SEQ ID NOs: 3 to 48 are employed in context of this invention, i.e. in the prepation of a pharmaceutical composition. Nucleic acid molecules encoding amino acid molecules/fragments as shown in SEQ ID NOs : 3 to 48 are easily obtainable by the skilled artisan, and may be produced, in accordance with the genetic code, by, inter alia, recombinant techniques, by chemical synthesis or by isolation from natural sources. However, the invention is not limited to the use of (antigenic) fragments shown in SEQ ID NOS: 3 to 48, but also relates to peptides which are highly homologous to the peptides shown in any one of said SEQ ID NOs. Most preferably, said homologous peptides are at least 80% more preferably at least 90% homologous to the corresponding peptide shown in any one of SEQ ID NO: 3 to 48. Accordingly in a peptide comprising 10 amino acids and shown in table 1 appended hereto, an exchange of one or two amino acids are envisaged to be still useful in accordance with this invention. From the appended examples, the person skilled in the art is provided with means and methods to test whether modified and or homologous peptides to the peptides shown in any one of SEQ ID NOs: 3 to 48 are useful in the preparation of a pharmaceutical composition, in particular of a vaccine and/or as an immunogenic composition. The invention further relates to a pharmaceutical composition comprising at least one nucleic acid molecule/polynucleotide coding for any one of the polypeptides as defined herein,

the antigenic fragment as defined herein and/or a fusion protein as defined herein. Said nucleic acid molecules/polypeptides are particularly useful in DNA-vaccination approaches as documented in the appended examples.

The nucleic acid molecule of the invention may be DNA such as cDNA or RNA such as mRNA. Additionally, the nucleic acid molecule of the invention may be PNA. Its origin may be natural, synthetic or semisynthetic or it may be a derivative, such as said peptide nucleic acid (Nielsen, Science 254 (1991), 1497-1500). Furthermore, said nucleic acid molecule may be a recombinantly produced chimeric nucleic acid molecule comprising any of the aforementioned nucleic acid molecules either alone or in combination. Preferably, said nucleic acid molecule is part of a vector. Said vector may also be compared in the pharmaceutical composition of the invention.

Such vectors may be, e.g., a plasmid, cosmid, virus, bacteriophage or another vector used e.g. conventionally in genetic engineering, and may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions.
Furthermore, the vectors may, in addition to the nucleic acid sequences to be employed in the invention, comprise expression control elements, allowing proper expression of the coding regions in suitable hosts. Such control elements are known to the artisan and may include a promoter, translation initiation codon, translation and insertion site for introducing an insert into the vector. Preferably, the nucleic acid molecule of the invention is operatively linked to said expression control sequences allowing expression in eukaryotic or prokaryotic cells.

Control elements ensuring expression in eukaryotic and prokaryotic cells are well known to those skilled in the art. As mentioned above, they usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally-associated or heterologous promoter regions. Possible regulatory elements permitting expression in for example mammalian host cells comprise the CMV- HSV thymiakine kinase promoter, SV40, RSV-promoter (Rous

sarcoma virus), human elongation factor 1α-promoter, CMV enhancer or SV40-enhancer. For the expression in prokaryotic cells, a multitude of promoters including, for example, the tac-lac-promoter or the trp promoter, has been described. Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pRc/CMV, pcDNA1, pcDNA3 (In-vitrogene), pSPORT1 (GIBCO BRL), or prokaryotic expression vectors, such as lambda gt11. Beside the nucleic acid molecules of the present invention, the vector may further comprise nucleic acid sequences encoding for secretion signals. Such sequences are well known to the person skilled in the art. Furthermore, depending on the expression system used leader sequences capable of directing the protein/(poly)peptide to a cellular compartment may be added to the coding sequence of the nucleic acid molecules of the invention and are well known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a protein thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including a C- or N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the proteins, antigenic fragments or fusion proteins of the invention may follow. In accordance with this invention, the vector can also comprise regulatory regions from pathogenic and eukaryotic organisms. Transcriptional targeting of genes and transcriptional regulatory sequences like CD molecules [Immunology. 103 (2001), 351] are an important way to control gene expression and can direct high level expression in vivo and in vitro. Expression can additionally be enhanced by non-coding sequences that modify cis-acting regulatory domains, including the Kozak consensus element, the gene-end signal, and the mRNA 5'-untranslated sequence. To improve the stability of the vector, an internal ribosome

entry site (IRES) can be used for co-expression instead of a second subgenomic promoter to ensure plasmid stability and integrity by elimination of homologues regions. A growth factor-like domain may be used to stabilize and target antigen expression.

Furthermore, said vector may also be a gene transfer or targeting vector. Gene therapy, which is based on introducing therapeutic genes (for example for vaccination) into cells by ex-vivo or in-vivo techniques is one of the most important applications of gene transfer. Suitable vectors, vector systems and methods for in-vitro or in-vivo gene therapy are described in the literature and are known to the person skilled in the art; see, e.g., Giordano, Nature Medicine 2 (1996), 534-539; Schaper, Circ. Res. 79 (1996), 911-919; Anderson, Science 256 (1992), 808-813, Isner, Lancet 348 (1996), 370-374; Muhlhauser, Circ. Res. 77 (1995), 1077-1086; Wang, Nature Medicine 2 (1996), 714-716; WO 94/29469; WO 97/00957, Schaper, Current Opinion in Biotechnology 7 (1996), 635-640 or Verma, Nature 389 (1997), 239-242 and references cited therein. The nucleic acid molecules and vectors to be employed in the present invention, in particular for the preparation of a pharmaceutical composition as described herein above may be designed for direct introduction or for introduction via liposomes, or viral vectors (e.g. adenoviral, retroviral) into the cell. Additionally, a baculoviral system can be used as eukaryotic expression system for the nucleic acid molecules of the invention. In addition to recombinant production, fragments of the protein, the fusion protein or antigenic fragments to be employed in accordance with this invention may be produced by direct peptide synthesis using solid-phase techniques (cf Stewart et al. (1969) Solid Phase Peptide Synthesis, WH Freeman Co, San Francisco; Merrifield, J. Am. Chem. Soc. 85 (1963), 2149-2154). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City CA) in accordance with the instructions provided by the manufacturer. Various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

Most preferably, the invention relates to a pharmaceutical composition comprising a host cell which comprises the polynucleotide or the vector as defined herein. Said host cell may be, inter alia, a dendritic cell; see also Tascon, Immunology 99 (2000), 473-480. Yet, not only eukaryotic cells are envisaged as host cells, but also prokaryotic cells, like a Mycobacterium cell, Salmonella-cells, Listria-cells, Lactobacillus-cells, Lactococcus-cells, Yersinia-cells or Shigella-cells. The vector comprised in the pharmaceutical composition of the invention may also be a viral vector. Such vectors, e.g. for gene transfer into antigen presenting cells are, inter alia, described in Monahan, Curr. Opin. Mol. Ther. 5 (1999), 558-564. Yet, further vectors are envisaged which comprise but are not limited to, Vaccinia-, Semliki Forest Virus-, Sendai Virus-, Rubella Virus (RUB)- or Influenza-, Poliovirus – Replicon-Vectors.

The invention in addition relates to a composition comprising at least one nucleic acid molecule/polynucleotide as defined herein. Said composition is useful, inter alia, for medical and diagnostic purposes, in particular, for pharmaceutic and vaccination purposes.

Moreover, the invention relates to pharmaceutical compositions comprising an antibody or a fragment or a derivative thereof directed against the polypeptide, the antigenic fragment, the nucleic acid molecule or the fusion protein as defined herein. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric or single chain antibodies or fragments or derivatives of such antibodies. As documented in the appended examples, Rv1511 or fragments thereof is surprisingly capable of eliciting a potent immune response. Accordingly, in context of this invention, it is envisaged that antibodies directed against the antigenic fragment, the nucleic acid molecule or the fusion protein as defined herein are employed in medical settings, for example in preventive or therapeutic immunization approaches through passive immunizations. Preferred are antibodies generated in non-human animals, like, mouse, rat, or swine. However, particularly preferred is the generation of antibodies for passive immunization in horse, camel and goat.

The general methodology for producing antibodies is well-known and has been described in, for example, Köhler and Milstein, Nature 256 (1975), 494 and reviewed in J.G.R. Hurrel, ed., "Monoclonal Hybridoma Antibodies: Techniques and Applications", CRC Press Inc., Boco Raron, FL (1982), as well as that taught by L. T. Mimms et al., Virology 176 (1990), 604-619. Furthermore, the generation of polyclonal antibodies/antisera is well known in the art. As stated above, in accordance with the present invention the term "antibody" relates to monoclonal or polyclonal antibodies. Antibody fragments or derivatives comprise $F(ab')_2$, Fab, Fv, scFv fragments, chimeric antibodies, humanized antibodies, CDR-grafted antibodies and the like; see, for example, Harlow and Lane, "Antibodies, A Laboratory Manual", CSH Press 1988, Cold Spring Harbor, NY or in Riechmann (1988), Nature 332, 323-327, Whittle (1989), J. Cell. Biochem 13A, p.96, Queen (1989), PNAS 86, 10029-10033, WO 86/01533, WO 90/07861, . Furthermore, in accordance with the present invention, the derivatives of antibodies can be produced by peptidomimetics. It is also envisaged that antibodies (or fragments thereof) are further optimized. Optimization protocols for antibodies are known in the art. These optimization protocols comprise, inter alia, CDR walking mutagenesis as disclosed and illustrated herein and described in Yang (1995), J. Mol. Biol. 25, 392-403; Schier (1996), J. Mol. Biol. 263, 551-567; Barbas (1996), Trends. Biotech 14, 230-34 or Wu (1998), PNAS 95, 6037-6042; Schier (1996), Human Antibodies Hybridomas 7, 97; Moore (1997), J. Mol. Biol. 272, 336.

"Panning"-techniques are also known in the art, see, e.g. Kay (1993), Gene 128, 59-65. Furthermore, publications like Borrebaeck (1995), "Antibody Engineering", Oxford University, 229-266; McCafferty (1996), "Antibody Engineering", Oxford University Press; Kay (1996), A Laboratory Manual, Academic Press provide for optimization protocols which may be modified in accordance with this invention. Such production methods are well known in the art and can be applied by the person skilled in the art without further ado.

In this context, the invention also relates to a method for the production of an antibody or a fragment thereof directed against a polypeptide as defined in herin above, i.e. to Rv1511 or (a) fragment(s) thereof comprising the steps of

(a) administering to an non-human animal a peptide as shown in any one SEQ ID NOs. 3 to 48, an isolated or recombinantly produced RV1511 or a fragment thereof or a nucleic acid molecule encoding a peptide as shown in any one SEQ ID NOs. 3 to 48 or a nucleic acid molecule encoding Rv1511 or a fragment therof; (b) eliciting an immune response in said non-human animal; and (c) isolating antibodies or fragments thereof generated in said non-human animal. Similarly, the invention relates to a method for the production of an antibody or fragments thereof directed against a polypeptide as herein, i.e. Rv1511, comprising the steps of (a) administering to an non-human animal a peptide as shown in any one SEQ ID NOs. 3 to , an isolated or recombinantly produced RV1511 or a fragment thereof or a nucleic acid molecule encoding a peptide as shown in any one SEQ ID NOs. 3 to 48 or a nucleic acid molecule encoding Rv1511 or a fragment thereof, (b) eliciting an immune response in said non-human animal; (c)isolating from said non-human animal a producing said antibody; (d)fusing said isolated cell to an immortalized cell; (e)culturing the resulting hybridoma cell; and (f) isolating from the culture medium the antibody secreted.

The method of production of antbodies as disclosed herein may also further comprise the modification of the isolated antibody and/or the production of a modified antibody molecule or an antibody derivative. Said modifiactions may comprise the isolation and determination of the corresponding CDR regions of the antibodies as well as the generation of humanized or chimeric antibodies by techniques known in the art. Furthermore, it is envisaged that antibodies or fragment(s) thereof may be labeled, preferably detectably labeled. Labeled antibodies or fragments thereof are particularly useful for diagnostic purposes.

Furthermore, the invention relates to a composition comprising at least one antibody, a fragment or a derivative thereof as defined above. Such antibodies, fragments or derivatives can be used for diagnostic or for pharmaceutical purposes, i.e. for the treatment of Mycobacteria-induced diseases, the vaccination against these pathogens or for the detection of a mycobacterial infection or the detection of RV1511 or (a) fragment(s) thereof. The composition of the invention comprising the

above defined antibodies, derivatives or fragments thereof is particularly useful in passive immunization approaches.

The invention also relates to a pharmaceutical composition further comprising, optionally, a pharmaceutically acceptable carrier.

The invention also relates to a pharmaceutical composition comprising a recombinant bacterial host cell of an avirulent strain or a vaccine strain which comprises at least one polynucleotide as defined herein above. Said recombinant bacterial host cell of an avirulent strain may be, but is not limited to, M. bovis, M. bovis BCG. Further envisaged recombinant host cells, in particular bacterial host cells are described herein above and comprise, but are not limited to, Mycobacterium-cells, Salmonella-cells, Listeria-cells, Lactobacillus-cells, Lactococcus-cells, Yersinia-cells and Shigella-cells.

Furthermore, the invention provides for a pharmaceutical composition as defined herein above, wherein said recombinant cell comprises at least one further nucleic acid molecule/polynucleotide encoding a peptide or polypeptide capable of eliciting and/or triggering an immune response in a mammal, preferably in a human.

The invention additionally relates to a pharmaceutical composition, wherein the peptide or polypeptide capable of eliciting and/or triggering an immune response is selected from the group consisting of auto-antigens, tumor-antigens, virus-antigens, parasite-antigens, bacterial-antigens and/or immunogenic fragments thereof. Such antigens are described herein above.

It is preferred that the pharmaceutical composition of the invention comprises a host cell which is capable of expressing at least one recombinant nucleic acid molecule as defined herein. As mentioned herein above, said host cell may be a bacterial host cell. Preferably, said bacterial host cell is selected from the group consisting of Salmonella ssp., M. bovis, M. bovis BCG, Lactobacillus ssp., Lactococcus ssp., or may also comprise host cells like further Mycobacterium-cells, Listeria-cells, Yersinia-cells as well as Shigella-cells.

In a preferred embodiment of the present invention the pharmaceutical composition as defined herein is a vaccine and/or said pharmaceutical composition has use as an immunogenic composition.

Vaccines may be prepared, inter alia, from one or more proteins, derivatives of the proteins, nucleic acid molecules, fusion proteins, antigenic fragments or antibodies, fragments of said antibodies or derivatives of the antibodies as defined herein.
For example, nucleic acid molecules/polynucleotides may be used for gene vaccination or as DNA vaccines the appended examples illustrate how DNA vaccines may be prepared and employed. Routes for administration of gene/DNA vaccines are well known in the art and DNA vaccination has been successfully used to elicit alloimmune, anti-tumor and antiidiotype immune responses (Tighe M. et al., Immunology Today 19 (1998), 89-97). Moreover, inoculation with nucleic acid molecules/DNA has been found to be protective in different modes of disease (Fynan, Proc. Natl. Acad. Sci. U.S.A. 90 (1993), 11478-11482; Boyer, Nat. Med. 3 (1997), 526-532; Webster, Vaccine 12 (1994), 1495-1498; Montgomery et al., DNA Cell Biol. 12 (1993), 777-783; Barry, Nature 311 (1995), 632-635; Xu and Liew, Immunology 84 (1995), 173-176; Zhoug, Eur. J. Immunol. 26 (1996), 2749-2757; Luke, J. Inf. Dis. 175 (1997), 91-97; Mor, Biochem. Pharmacology 55 (1998), 1151-1153; Donelly, Annu. Rev. Immun. 15 (1997), 617-648; MacGregor, J. Infect. Dis. 178 (1998), 92-100).

An approach currently being applied in scientific and preclinical studies is the genetic immunization with naked DNA (62). The observations that plasmid DNA could directly transfect eukaryotic cells in vivo lead to the use of DNA plasmids to induce immune responses by direct injection into animals (60). DNA vaccines encode eukaryotic vectors expressing antigens in the vaccinated host (59) and therefore elicit humoral and cellular immune responses. Most often the DNA is injected as an aqueous solution intramuscular (i. m.) (72) or is administered by particle bombardment of skin with DNA coated onto gold particles using a gene gun (g. g.). Vaccination via the i. m. route is considered to induce a Th1 immune response characterized by interferon gamma (IFN-γ) secreting T-cells and the

presence of cytotoxic T-lymphocytes (CTL) (69). In contrast, g. g. vaccination preferentially induces a Th2 biased response with interleukin-4 (IL4) producing T-cells and antibodies of the IgG1 isotype (63). DNA vaccines may have a special role in preventing bacterial (70) and viral infections (68), and in tumor therapy (69). The World Health Organization has started a global project for designing effective control strategies and to determine treatment levels for eradication of tuberculosis (58). Results were already published for i. m. DNA vaccination using hsp65 (71), esat-6 (65), Ag85A and Ag85B (66). Ag85 is so far the best characterized tuberculosis DNA vaccine successfully applied in different experiments with a well characterized immune response(61, 64, 66, 67, 73).

Preferably, the polynucleotide to be employed as DNA-vaccine comprises the coding region for at least one, preferably at least two, more preferably at least three antigenic fragments of Rv1511 or of homologous proteins capable of, inter alia, eliciting an immune response preferably in a mammal, most preferably in a human.

The proteins, nucleic acid molecules, fusion proteins, antigenic fragments or antibodies, fragments or derivatives of said antibodies to be used in the pharmaceutical composition of the invention as a vaccine may be formulated e.g. as neutral or salt forms. Pharmaceutically acceptable salts, such as acid addition salts, and others, are known in the art. Vaccines can be, inter alia, used for the treatment and/or the prevention of an infection with pathogens and are administered in dosages compatible with the method of formulation, and in such amounts that will be pharmacologically effective for prophylactic or therapeutic treatments.

Proteins, protein fragments and/or protein derivatives used as vaccines are well known in the art (see, e.g. Cryz, "Immunotherapy and Vaccines", VCH Weinheim (1991); Paul (1989), loc. cit.). Furthermore, it has been shown that even intracellular enzymes of bacterial pathogens can act as antigenic entities which provide immunological protection (Michetti, Gastroenterology 107 (1994), 1002; Radcliff, Infect. Immun. 65 (1997), 4668; Lowrie, Springer Semin. Immunopathol. 19 (1997), 161)

A vaccination protocol can comprise active or passive immunization, whereby active immunization entails the administration of an antigen or antigens (like the compositions of the present invention or proteins, nucleic acid molecules, fusion proteins, antigenic fragments or antibodies, fragments of said antibodies or derivatives of the antibodies of the present invention) to the host/patient in an attempt to elicit a protective immune response. Passive immunization entails the transfer of preformed immunoglobulins or derivatives or fragments thereof (e.g., the antibodies, the derivatives or fragments thereof of the present invention) to a host/patient. Principles and practice of vaccination and vaccines are known to the skilled artisan, see, for example, in Paul, "Fundamental Immunology" Raven Press, New York (1989) or Morein, "Concepts in Vaccine Development", ed: S.H.E. Kaufmann, Walter de Gruyter, Berlin, New York (1996), 243-264. Typically, vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in or suspension in liquid prior to injection also may be prepared. The preparation may be emulsified or the protein may be encapsulated in liposomes. The active immunogenic ingredients often are mixed with pharmacologically acceptable excipients which are compatible with the active ingredient. Suitable excipients include but are not limited to water, saline, dextrose, glycerol, ethanol and the like; combinations of these excipients in various amounts also may be used. The vaccine also may contain small amounts of auxiliary substances such as wetting or emulsifying reagents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. For example, such adjuvants can include aluminum compositions, like aluminumhydroxide, aluminumphosphate or aluminumphosphohydroxide (as used in "Gen H-B-Vax®" or "DPT-Impfstoff Behring"), N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-DMP), N-acetyl-nornuramyl-L-alanyl-D-isoglutamine (CGP 11687, also referred to as nor-MDP), N-acetylmuramyul-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'2'-dipalmitoyl-sn-glycero-3-hydroxphaosphoryloxy)-ethylamine (CGP 19835A, also referred to as MTP-PE), MF59 and RIBI (MPL + TDM + CWS) in a 2% squalene/Tween-80® emulsion. Further adjuvants may comprise DNA or oligonucleotides, like, inter alia, CpG-containing motifs (CpG-oligonucleotides; Krieg, Nature 374 (1995), 546-549; Pisetsky, An. Internal. Med. 126 (1997), 169-171).

The vaccines usually are administered by intravenous or intramuscular injection. Furthermore, it is envisaged that the vaccines described herein are administered by mucosal (e.g. intranasal, oral, subcutaneaous) or transdermal routes. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include but are not limited to polyalkylene glycols or triglycerides. Oral formulation include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions may take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 10% to about 95% of active ingredient, preferably about 25% to about 70%.

Vaccines are administered in a way compatible with the dosage formulation, and in such amounts as will be prophylactically and/or therapeutically effective. The quantity to be adminstered generally is in the range of about 5 micrograms to about 250 micrograms of antigen per dose, and depends upon the subject to be dosed, the capacity of the subject's immune system to synthesize antibodies, and the degree of protection sought. Precise amounts of active ingredient required to be administered also may depend upon the judgment of the practitioner and may be unique to each subject. The vaccine may be given in a single or multiple dose schedule. A multiple dose is one in which a primary course of vaccination may be with one to ten separate doses, followed by other doses given at subsequent time intervals required to maintain and/or to reinforce the immune response, for example, at one to four months for a second dose, and if required by the individual, a subsequent dose(s) after several months. The dosage regimen also will be determined, at least in part, by the need of the individual, and be dependent upon the practitioner's judgment. It is contemplated that the vaccine containing the immunogenic compounds of the invention may be administered in conjunction with other immunoregulatory agents, for example, with immunoglobulins, with cytokines or with molecules which optimize antigen processing, like listeriolysin.

In accordance with this invention, the vaccines described herein may be used for prophylactic as well as for therapeutic purposes.

The invention, accordingly, also relates to a pharmaceutical composition as defined herein which is a living vaccine suitable for administration to a mucosal surface via the parenteral route.

The pharmaceutical composition may comprise the herein defined proteins, the fusion proteins, antigenic fragments and/or antibodies (or their fragments or derivatives) of the invention, either alone or in combination. The pharmaceutical composition of the present invention may be used for effective therapy of infected humans and animals for vaccination purposes and/or prevention.

It is of note that the pharmaceutical composition of the present invention may, additionally, comprise further antigenic determinants or determinants capable of eliciting an immune response or reaction. Said determinants may comprise other differentially expressed polypeptides, like differentially expressed polypeptides as described in WO 00/44392. These further determinants may comprise, but are not limited to proteins/polypeptides which are differentially expressed in M. tuberculosis H37Rv/Erdman as compared to M. bovis BCG. Such proteins comprise Rv3710, Rv1392, Rv0952, Rv2971, Rv0068, Rv0120c, Rv2883c, Rv1463, Rv1856c, Rv2579, Rv3275c, Rv2557, Rv3407, Rv3881c, Rv2449c, Rv0036c, Rv2005c or Rv3676. Functional fragments of said polypeptides capable of eliciting an immunologic reaction or being antigenic fragments, are also envisaged to be comprised, preferably additionally comprised in the (pharmaceutical) composition of this invention. It is understood that also polynucleotides/nucleic acid molecules encoding said differentially expressed polypeptides or functional fragments thereof be comprised in said (pharmaceutical) composition.

The above-mentioned differentially expressed polypeptides may also be linked to the polypeptides being Rv1511 or being derived from Rv1511. Such linkage may comprise fusion proteins as well as polynucleotide constructs comprising coding-sequences for Rv1511 or fragments thereof in combination with coding sequences

of the above-mentioned additional antigenic fragments. Such polynucleotides are particularly useful in DNA-vaccination approaches.

The pharmaceutical composition of the present invention may further comprise a pharmaceutically acceptable carrier, excipient and/or diluent. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 10 mg per dose; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. If the regimen is a continuous infusion, it should also be in the range of 1 µg to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. The compositions of the invention may be administered locally or systemically. Administration will generally be parenterally, e.g., intravenously. The compositions of the invention may also be administered directly to the target site, e.g., by biolistic delivery to an internal or external target site or by catheter to a site in an artery. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or

suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents such as interleukins, interferons and/or CpG-containing DNA stretches, depending on the intended use of the pharmaceutical composition.

The invention also provides for a recombinant bacterial host cell of an avirulent strain or a vaccine strain which comprises at least one polynucleotide as defined herein. Said recombinant (bacterial) host cell is particularly useful as a vaccine strain against mycobacterial-induced diseases, in particular against TB.

Preferably said recombinant cell comprises at least one further nucleic acid molecule encoding a peptide or polypeptide capable of eliciting and/or trigger an immune response in a mammal. Said peptide or polypeptide capable of eliciting and/or trigger an immune response is preferably selected from the group consisting of autoantigens, tumor-antigens, virus-antigens, parasite-antigens, bacterial-antigens and/or immunogenic fragments thereof.

Preferably, said host cell is capable of expressing said polynucleotide and said at least one further recombinant nucleic acid molecule.

This invention also relates to a fusion protein as defined herein above. Said fusion protein is particularly useful in vaccination protocols against bacteria-induced diseases, in particular mycobacteria-induced disease.

The invention also relates to a polynucleotide encoding for the fusion protein as defined herein. Said polynucleotide is preferably comprised in a vector, e.g. an expression vector as defined herein.

Furthermore, the invention provides for a method for the production of a vaccine against a virulent strain of the genus Mycobacterium comprising the step of combining a recombinant polypeptide as defined herein with a pharmaceutically acceptable carrier. Said polypeptide may not comprise expressed Rv1511 and/or fragments thereof but may also be a fusionprotein/polypeptide as defined herein. Preferably said method additionally comprises the recombinant expression of a polynucleotide as defined herein for recombinantly producing said polypeptide. Accordingly, the polypeptide to be used in said method may be recombinantly produced.

The invention also provides for a method for the production of a vaccine against a virulent strain of the genus Mycobacterium comprising the step of combining a peptide as shown in any one of SEQ ID NOs: 3 to 48 (or as depicted in the appended table 1)with a pharmaceutically acceptable carrier.

The invention also provides for a method for the production of a vaccine against a virulent strain of the genus Mycobacterium comprising the step of combining a polynucleotide or a vector as defined herein with a biologically and/or pharmaceutically acceptable carrier, wherein said polynucleotide or said polynucleotid comprised in said vector is placed under the control of an expression control sequence.
Useful expression control sequences are defined herein above but further, specific expression control sequences comprise tissue plasminogen activator (tpa), epidermal growth hormone (EGF), human growth hormone (HGH).

Furthermore, the invention relates to the use of a polynucleotide or a vector as defined herein above for the preparation of a vaccine for vaccination against a virulent strain of the genus Mycobacterium or against a mycobacterium-induced disease. In addition, it is envisaged that a peptide as depicted in any one of SEQ ID NOs: 3 to 48 is usedfor the preparation of a vaccine for the vaccination against a virulent strain of the genus Mycobacterium or against a mycobacterium-induced disease. Most preferably, the peptide to be employed for the preparation of said vaccine is selected from the group consisting of SEQ ID NO: 13, 20, 23, 24, 29, 35

or 42. Said vaccination may be employed to prevent as well as to treat such a disease. Preferably said mycobacterium-induced disease is selected from the group consisting of tuberculosis, tropical skin ulcer, ulceration, absess, granulomatous (skin) disease, pulmonary disease, lymphadenitis, and cutaneous and disseminated disease.
However, the use is not restricted to mycobacteria-induced diseases in humans but also in the prevention and/or treatment of animal diseases, like bovine tuberculosis.

As documented in the appended examples, peptides derived from RV1511 are very potent in eliciting immune response. Accordingly, the present invention also provides for specific, isolated peptides derived from Rv1511 and shown in table 1. Therefore, the invention relates in a further embodiment to an isolated peptide selected from the group consisting of a peptide as depicted in table 1 or in any one of SEQ ID NOs: 3 to 48 (in particular peptides as shown in SEQ ID NO: 13, 20, 23, 24, 29, 35 or 42 or an isolated peptide which is at least 80% homologous to a peptide as shown in SEQ ID NOs: 3 to 48. The invention also provides for nucleic acid molecules encoding the peptides disclosed herein, in particular peptides SEQ ID NO: 13, 20, 23, 24, 29, 35 or 42

The Figures show:

Figure 1: Sectors from two-dimensional electrophoretic patterns of total cell proteins of attenuated (**A**: *M. bovis* BCG Chicago; **B**: *M. bovis* BCG Copenhagen) and virulent (**C**: *M. tuberculosis* H37Rv; **D**: *M. tuberculosis* Erdman) mycobacterial strains. The spots 2_25 of *M. tuberculosis* H37Rv and 4_25 of *M. tuberculosis* Erdman had no counterparts in the protein patterns of the attenuated *M. bovis* BCG strains and were identified as probable GDP-D-mannose dehydratase (encoded by the ORF Rv1511) of *M. tuberculosis* H37Rv.

Figure 2: Plasmid map of the DNA vector pCMVtpaRv1511.

The DNA vector pCMVtpaRv1511 is a constitutive mammalian expression plasmid optimized for heterologous expression of antigens. Expression of Rv1511 is driven by the human cytomegalovirus (CMV) immediate early gene promoter downstream of the mycobacterial antigen Rv1511. The antigen is proximally fused to the human tissue plasminogen activator (tpa) leader sequence following an *Eco*RI restriction endonuclease site and translation begins at the first start codon (AUG) distal an untranslated sequence derived from tpa. Downstream the Rv1511 antigen a polyadenylation signal and the bovine growth hormone (BGH) terminator is followed by a deleted pUC backbone, containing the Ampicillin resistence gene.

Figure 3: Vaccination results.

The correlate of protection was measured by colony forming unit (CFU) analysis of lungs from DNA vaccinated and control mice after aerosol challenge with virulent *M. tuberculosis* H37Rv. Four different groups of mice are depicted in the graph: circles with dotted connecting line represent the positive control group immunized with *M. bovis* BCG Copenhagen; filled squares with solid connection line represent pCMVtpaRv1511 DNA vaccinated group; triangles with dash-dotted connection line represents the negative pCMVtpa plasmid vector control group and crosses with a dashed connection line represents the naive control group. The bacterial load of lungs was determined at day 14, 28 and 60 after challenge infection of approximately 200 bacilli via the aerosol route. The standard error of the mean is given as error bar. Statistical significance between the pCMVtpaRv1511 vaccinated group and the pCMVtpa negative control groups for each time point was determined by the Mann-Whitney two-tailed statistical test and the medians are displayed as significant by asterisks for a P value below 0.05.

Figure 4-5: Further vaccination results and Protection against tuberculosis by DNA vaccination

Protection against tuberculosis by DNA vaccination. BALB/c mice were immunised three times with 100µg of the DNA construct. Mice were challenged with TB (200 bacteria per lung) 21 days after the last boost by aerosol. Bars represent the mean +/- SD of the CFU counts of 7 mice per group.

Figure 6-9: IFN-γ ELISpot assay on day 14 after the last boost. BALB/c mice were immunised three times with 100 µg of the DNA constructs. Potential peptide epitopes were selected using a new MHC class I prediction program. For Rv 1511 seven peptides were generated (n.b. for the others two peptides). Bars represent the mean +/- SD of the spot counts of 3 mice per group.

Figures 7-9: IFN-γ ELISpot assay on days 7, 14, 28 and 60 after the last boost. BALB/c mice were immunised three times with 100 µg of the DNA constructs. Potential peptide epitopes were selected using a new MHC class I prediction program. For Rv 1511 seven peptides were generated (n.b. for the others two peptides). Bars represent the mean +/- SD of the spot counts of 3 mice per group.

Figure 10: Western-blot of a 10% SDS-PAGE.
Western-blot analysis with blood sera from Rv1511 immunized mice. In the first line *M. tuberculosis* H37Rv (lab strain) lysate, in the second line lysate from *M tuberculosis* Beijing (clinical isolate).

Figure 11: Determination of IgG isotypes.
IgG isotypes were measured from mice vaccinated with Rv1511 or control vector. For additional control blood was taken from naive mice. IgG1 and IgG2a isotypes were only determined from mice vaccinated with Rv1511. The figure shows ELISA-resultes with sera from Rv1511 immunised mice. Plates were coated with lysate from M.tb or recombinant Rv1511 from *E.coli*. Sera dilution of 1/50.

The Examples illustrate the invention:

Example I: 2DE-Analysis

2-DE protein patterns of whole cell preparations of 2 virulent (*M. tuberculosis* strains H37Rv and Erdman) and 2 attenuated (*M. bovis* BCG substrains Chicago and Copenhagen) mycobacterial strains were compared. H37Rv and Erdman are the most commonly used laboratory strains of *M. tuberculosis*. Chicago (= Tice) and Copenhagen (= Danish strain 1331) are representative and well characterized strains of the *M. bovis* BCG vaccine. The Copenhagen strain belongs to a group of 4 vaccine strains that account for more than 90% of the BCG vaccines currently in use worldwide [30].

Following materials and methods were employed:

Materials

Two virulent strains of *M. tuberculosis*, H37Rv (from our strain collection) and Erdman (kind gift of B. Bloom; Albert Einstein College of Medicine, Bronx, NY), and 2 attenuated strains of *M. bovis* BCG, Chicago (= Tice; ATTC No. 27289) and Copenhagen (= Danish strain 1331; Statens Serum Institute, Copenhagen, Denmark) were investigated. The 2-DE equipment was purchased from WITA (Teltow, Germany). Trypsin used for in-gel digestion of proteins was obtained from Promega (Madison, WI, USA). Other chemicals were purchased from Bio-Rad (Munich, Germany) or Merck (Darmstadt, Germany). Peptide solutions were concentrated by a speed vac concentrator (Savanta, Hicksville, NY, USA). MALDI-MS was carried out with a time-of-flight MALDI mass spectrometer Voyager Elite (Perseptive, Framingham, MS, USA) with delayed extraction. Nano-ESI-MS/MS was performed with a Q-Tof mass spectrometer (Micromass, Manchester, UK).

Sample preparation and two-dimensional electrophoresis

Cellular proteins of *M. tuberculosis* H37Rv were prepared from mycobacterial whole cell lysates as described [26]. In short, mycobacteria were grown in Middlebrook medium for 6-8 days to a cell density of 1-2 x 10^8 cells per ml, cells were washed and sonicated in the presence of proteinase inhibitors, and proteins were treated with 9 M urea, 70 mM dithiothreitol and 2% Triton X-100 to obtain completely denatured and reduced proteins. The separation of proteins by 2-DE was performed as described [31], using a combination of carrier ampholyte IEF and SDS-PAGE. The gel size was 22 x 30 cm. IEF was performed in rod gels containing 9 M urea, 3.5% acrylamide, 0.3% piperazine diacrylamide and a total of 4% carrier ampholytes pH 2-11. Protein samples were applied at the anodic side of the IEF gels and focused under non-equilibrium pH gradient electrophoresis conditions (8 870 Vh). For analytical and preparative investigations 0.75 or 1.5 mm thick gels were used, respectively. For analytical investigations 100 µg of protein sample were applied. For preparative experiments we used up to 900 µg of protein sample. SDS-PAGE was performed in gels containing 15% acrylamide using the IEF gels as stacking gels. Following electrophoresis the gels were stained. For analytical and preparative investigations proteins were visualized as described by either silver staining or Coomassie Brilliant Blue G250 (CBB G250) staining [32-33].

Evaluation of variant spots

The evaluation of variant spots was performed visually and restricted to spots exclusively present in the virulent mycobacterial strains. Three individual sample preparations were prepared per strain. Two 2-DE runs were performed per preparation. To elucidate potential variations between the virulent and attenuated mycobacterial strains, 2 patterns of independently prepared samples of *M. bovis* BCG Chicago and *M. bovis* BCG Copenhagen each were compared individually with 2 patterns of *M. tuberculosis* H37Rv. Variants that were detected in all these comparisons were regarded as potential variants between virulent and attenuated mycobacterial strains. Only differences confirmed by the comparison of all 2-DE patterns, i.e. 6 patterns per strain, were accepted as specific variations.

Mass spectrometry

The identification of proteins separated by 2-DE was performed as described using MALDI-MS and nano-ESI-MS/MS [26, 34]. Spots from preparative CBB G250-stained 2-DE gels were excised and proteins were digested in-gel with trypsin. Following digestion, the resulting peptides were freed of salt using a peptide collecting device as described [35]. Peptides were eluted from the peptide collecting device in 100 µl 60% ACN, 0.3% TFA and concentrated to a final volume of approximately 10 µl using a speed vac concentrator. Two µl of the concentrated solutions were mixed with 2 µl of an aqueous saturated alpha-cyano-4-hydroxy cinnamic acid solution in 30% ACN, 0.3% TFA. Two µl of the resulting solutions were applied to the sample template of the MALDI-MS equipment and air dried. Mass spectra were recorded in the reflectron mode of a time-of-flight MALDI mass spectrometer *Voyager Elite* (Perseptive, Framingham, MS, USA) with delayed extraction. Mass accuracy in the range of 30 ppm was obtained by internal calibration using 3 synthetic peptides with known molecular mass as internal markers. Proteins were identified by peptide mass mapping (PMM) [36-39] by searches in the protein database of the National Center for Biotechnology Information (NCBI) [40]. Searches were performed using the program MS-FIT [41], reducing the proteins of the NCBI-database to the mycobacterial proteins and a molecular mass range estimated from 2-DE ± 20% and allowing a mass tolerance of 0.1 Da. If no proteins matched the molecular mass window was extended. Partial enzymatic cleavages leaving 2 cleavage sites, acetylation of N-terminal end of proteins, pyroglutamate formation at N-terminal glutamine of peptides, oxidation of methionine and modification of cysteine by acrylamide were considered in the database searches. A protein was regarded as identified if the matched peptides covered at least 30% of the complete protein sequence. An assignment with a sequence coverage below 30% was only accepted, if at least the 3 main peaks of the mass spectrum were matched with a database sequence. In some cases, sequence support was required, to confirm the identity of a proposed protein or to establish protein identity. Sequence support was obtained by either PSD MALDI-MS [42] or ESI-MS/MS [43] performed with a Q-Tof mass spectrometer (Micromass,

Manchester, UK) as described [34]. Here the sequence tag method [44] was used to search the proteins in the NCBI-database.

Results:

Silver stained 2-DE protein patterns of whole cell preparations of mycobacteria encompassed about 1 800 distinct protein species. The exact number of spots depended on the amount of sample applied to the gels and the staining conditions. Silver stained 2-DE protein patterns of whole cell preparations of all mycobacterial strains examined in this study are presented in our 2-DE database [29]. The genomes of members of the *M. tuberculosis* complex, including the four mycobacterial strains investigated, are highly conserved [45]. Consistent with this, the vast majority of protein spots had counterparts with identical electrophoretic mobility in all mycobacterial strains investigated.

Although the 2-DE patterns of the mycobacterial strains were highly comparable, clear differences in the presence or absence of spots (±-variants) were also observed. Fifty-six spots were exclusively detected in the virulent mycobacterial strains. So far, 44 of these spots were analyzed by MS, of which 32 were identified. Twelve of these spots were identified as proteins encoded by genes, previously reported to be deleted in *M. bovis* BCG Chicago and Copenhagen [4, 12-16]. One of these spots was identified as probable GDP-mannose 4,6 dehydratase encoded by ORF Rv1511 located in the deletion region RD6 of the genome of *M. tuberculosis* H37Rv: a genomic region that is known to be missing in both, virulent wild-type *M. bovis* and attenuated *M. bovis* BCG. The used RD nomenclature is based on that proposed by Behr *et. al.* [4]. For illustration, the spots 2_25 of *M. tuberculosis* H37Rv and 4_25 of *M. tuberculosis* Erdman identified as probable GDP-mannose 4,6 dehydratase are depicted in Figure 1. Furthermore, the nucleotide sequence encoding for Rv1511 is depicted in SEQ ID NO: 1. The corresponding amino acid sequence is shown in SEQ ID NO: 2.

Example II: DNA vaccine encoding for Rv1511

The vaccine candidate Rv1511 was derived from proteome analysis and was found to be differentially expressed in virulent M. tuberculosis strains compared to M. bovis BCG; see Example I herein above. As will be demonstrated here, the DNA vaccine encoding for Rv1511 resulted in an constant protection over all observed/examined time points.

Animals

BALB/c mice were bred at the central animal facilities of the Max-Planck-Institute for Infection Biology at the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BGVV, Berlin, Germany). Animals were kept under specific pathogen-free conditions and fed autoclaved food and water ad libitum. In given experiments, female mice were used at 8 weeks of age. Groups of at least 5 mice were used in all experiments.

Bacterial strains and cultures

M. tuberculosis H37Rv and M. bovis Calmette-Guérin bacillus (BCG) Danish 1331 (Statens Serum Institute, Copenhagen, Denmark) were cultivated on Middlebrook 7H11 agar supplemented with OADC enrichment w/WR 1339 (Difco, Detroit, U.S.A.). Tuberculosis stocks for infection were grown to an cell density of approximately 10^8 cells per ml in Middlebrook 7H9 medium supplemented with Middlebrook ADC enrichment (Difco, Detroit, U.S.A.). All bacterial infection stocks were washed once with PBS w/o Ca and were maintained as glycerol stocks at -70°C.

Plasmid vaccine DNA construction

Construction of DNA vaccines can be divided into three steps. First the sequence of interest is amplified by polymerase chain reaction (PCR) with specifically adapted PCR primers. Second, the amplification product is subcloned into a PCR cloning vector and its sequence identity is verified. Third the confirmed sequence is excised and ligated with a specific eukaryotic expression vector comprising the DNA vaccine.

The M. tuberculosis gene Rv1511 was cloned into the vaccine DNA plasmid vector pCMVtpa (Chiron-Behring, Emeryville, USA) under the control of the CMV promoter fused with the tissue plasminogen activator leader sequence, resulting in plasmid pCMVtpaRv1511. In an initial step the *Mycobacterium tuberculosis* gene Rv1511 was amplified from chromosomal DNA by gene specific oligonucleotides (primers pCMVtpaRv1511 5' 5' AGA TCT GTG AAG CGA GCG CTC ATC 3' (SEQ ID NO: 49) and pCMVtpaRv1511 3' 5' AGA TCT TGT CCG GCC GGC GAT 3' (SEQ ID NO: 50)) containing at their 5' ends an additional *BglII* cloning site (underlined sequence) to facilitate subsequent ligation into the DNA vaccine vector pCMVtpa. The amplification product encoding the terminal *BglII* cloning site and the complete *M. tuberculosis* gene Rv1511 was purified by agarose gel electrophoresis and subcloned into the SmaI restriction site of the commercial PCR cloning vector pUC18. The resulting plasmid carrying the subcloned PCR product was verified by restriction endonuclease digestion and by DNA sequencing. In a next step the inserted sequence was cleaved from the PCR cloning vector by *BglII* restriction endonuclease, specific for the incorporated 5' sites of the PCR product. The excised fragment was subsequently ligated with the *BamHI* predigested DNA vaccine vector pCMVtpa resulting in the final DNA vaccine construct pCMVtpaRv1511. The inserted sequence in the DNA vaccine encoding the *M. tuberculosis* antigen was confirmed by restriction endonuclease digestion and DNA sequencing. For use in DNA vaccination experiments, the DNA vaccine was purified as endotoxin free plasmid DNA from E. coli.

Intramuscular immunization

Seven female mice per group were immunized at the age of 6-8 weeks by intramuscular injection (i. m.). Before immunization mice were narcotized and their hind legs were shaved. The DNA vaccine was injected into the tibialis anterior muscles. A dose of 50 µg naked DNA in 50 µl PBS was injected into each tibialis anterior muscle. Mice were initially immunized at day –90 and were boosted twice in 30 day intervals. At day 0 vaccinated and control mice were challenged with M. tuberculosis.

BCG vaccination

Mice were vaccinated with 5×10^5 live M. bovis BCG at day –90 by i. v. injection into the tail vain. Mice were cured with rifampicin (100 mg/ml) and isoniazide (200 mg/ml) in the drinking water starting at day –60 over 21 days. Curing was verified by enumeration of bacterial titer at day –30 in spleen, liver and lung.

M. tuberculosis challenge

At day 0 all mice were challenged with 200 *M. tuberculosis* H37Rv bacilli by the use of an aerosol exposure system model 099C A4212 (Glas-Cole - Schütt Labortechnik, Göttingen, Germany). At day 1 after the challenge the infection dose was verified as CFU per lung. Mice were sacrificed at days 28 and 60 post challenge. Spleens and lungs were aseptically removed and organ homogenates were prepared. Bacterial counts were enumerated by plating serial dilutions of organ homogenates on Middlebrook 7H11 agar.

Correlates of protection in a murine model of tuberculosis

The *M. tuberculosis* DNA vaccine testing comprises three steps. The initial step of DNA vaccination is most often performed by intra muscular (i. m.) injection of naked DNA or by biolistic bombardment of DNA coated onto gold particles. Next, vaccinated and control animals are challenged by infection with virulent *M. tuberculosis*. The degree of protection is subsequently measured by enumeration of the bacterial load in different organs at various time points after the challenge.

Seven female mice per group were immunized at the age of 8 weeks by intramuscular (i. m.) injection. Before immunization, mice were anesthesized and their hind legs were shaved. The DNA vaccine was injected into the tibialis anterior muscles at a dose of 50 µg DNA in 50 µl PBS leading to a final concentration of 100 µg DNA per mouse for each immunization. Mice were boosted twice in 30 day intervals. Thirty days after the last boost immunization animals were challenged with 250 bacilli of virulent *M. tuberculosis* bacilli via the aerosol route using an inhalation exposure system. Groups of mice were sacrificed at various time points (days 14, 28 and 60) after challenge. Organs (spleen and lung) were aseptically removed and homogenates were prepared by grinding them in sealed bags. Bacterial counts were enumerated by plating serial dilutions of the organ homogenates on Middlebrook 7H11 agar. As negative control we used naive mice and the empty DNA vaccine

vector without recombinant insertion, animals immunized with the live attenuated vaccine strain *M. bovis* BCG served as positive control. Differences between groups were analyzed with the statistical Mann-Whitney test using two-tailed P values and a significant difference set at $P < 0.05$.

As depicted in Figure 3 three control groups and the pCMVtpaRv1511 DNA vaccine group were received by i. m. vaccination or i. v. immunization (*M. bovis* BCG Copenhagen live vaccine strain). Mice were challenged after vaccination via the aerosol route with virulent *M. tuberculosis* H37Rv and protection was measured by enumeration of viable tubercle bacilli in lungs. Naive mice and pCMVtpa i. m. treated mice served as negative control groups, whereas *M. bovis* BCG – Copenhagen immunized mice were used as positive control group. Mice vaccinated initially i. v. with a single dose of 5×10^5 viable *M. bovis* BCG Copenhagen were cured with rifampicin / isoniazide prior to challenge with M. tuberculosis. They were free of the vaccine strain as determined by CFU. Although no sterile protection was achieved, leading to complete eradication of *M. tuberculosis* bacilli after challenge in the model of murine tuberculosis, the live attenuated vaccine strain *M. bovis* BCG Copenhagen induced a high level of protection throughout the examined course of challenge infection. Additionally, the DNA vaccine pCMVtpaRv1511 caused a statistically significant protection with P values ≤ 0.05 as determined by the Mann-Whitney test as compared to the plasmid vector control over the examined time course after challenge. The protection induced by the DNA vaccine pCMVtpaRv1511 showed a constant CFU reduction of challenge tubercle bacilli with no decrease in protection at later time points after challenge. There was no difference between naive mice and the plasmid DNA control vector pCMVtpa treated mice, indicating that the protection induced by the DNA vaccine pCMVtpaRv1511 was not due to an unspecific effect of the DNA vector itself. Exacerbation of challenge infection with tubercle bacilli were determined for all groups, but only *M. bovis* BCG and pCMVtpaRv1511 lead to a significant reduction in CFU of lung.

Example III: Antigenic peptides of a M. tuberculosis DNA vaccine

The class I major histocompatibility complex (MHC I) presents 8-10 residue peptides to cytotoxic T lymphocytes (CTL). Most of these antigenic peptides are generated during protein degradation in the cytoplasm and are then transported into the endoplasmic reticulum where they are loaded onto MHC I molecules. We determined possible CTL antigenic peptide fragments derived from the M. tuberculosis DNA vaccine pCMVtpaRv1511 by a combination of different predictions. To this end MAPPP (MHC-I Antigenic Peptide Processing Prediction) was used, which predicts possible antigens as peptide fragments being processed by the proteasome and finally being presented by MHC I gene products on the cell surfaces. This combination allows the prediction of immunodominant CTL epitopes by joining the proteolytic activity of the proteasome and the antigen presentation via MHC I.

MAPPP first generates a probability for the cleavage of each possible peptide from a protein by proteasome activity. This probability is based on a statistic-empirical method. Peptides with the highest probabilities of proteasome processing are then given an additional score reflecting their ability of binding to MHC I molecules. This binding score employs coefficient tables. A combination of two independent predictions for proteasome processing and MHC I presentation increases the possibility to retrieve more precise predictions on MHC I presented immunodominant T- cell epitopes.

Cleavage prediction

For calculation of the cleavage probability of a specific epitope fragment from pCMVtpaRv1511 the probability of a cut after each of the residues within the sequence is determined first. By limiting the minimal probability for a single residue, a limitation of the number of resulting peptides was possible. After that, the cleavage probability for all possible fragments between two cut-sites within the right length was calculated. The flanking regions to the C-terminal and the N-terminal end of a fragment and the probabilities of their residues were also considered within the calculation. The program used the algorithms first implemented in FRAGPREDICT, a computer program for the prediction of proteasomal cleavage sites and proteolytic fragments [46] or alternatively the prediciton algorithms of PaProC [47].

MHC binding prediction

The prediction of antigens derived from pCMVtpaRv1511 presented by MHC class I molecules is based on a score table calculation for each subsequence generated by proteasomal prediction. An additional score was derived from a comparison with different matrices specific for individual MHC haplotypes (e.g. HLA-A201, H2Kb, H2Dd, H2Kd, H2Dd, H2Ld etc.) and different species, in particular, mouse, cattle (bovine) and most preferably in human. Each specific amino acid at a specific position within a subsequence was given a value. Depending on the algorithms selected, the values for potential epitopes of the correct length, normally between 8 to 10 amino acids, were then multiplied for BIMAS calculation [48] or added in case of SYFPEITHI [49] to determine the score for the subsequence. These values for different species and their distinct haplotypes used for calculation were pre-calculated and stored in static matrices. Yet, even if certain peptides in the appended table are linked to distinct haplotypes of certain species, i.e mouse, cattle or human, it is of note that said peptide/fragment of Rv1511 may also be employed in context of this invention in any other species.

An overall value was finally generated by combining one of the values for the proteasomal cleavage probability from FRAGPREDICT or PAPROC with the value for class I MHC binding score from BIMAS or SYFPEITHI with a limitation excluding C-terminal elongated peptides.

Antigenic peptides derived from Rv1511 as herein discussed are shown in the following appended table I.

Example IV: Detailed vaccination experiments

Further experiments were carried out, to allow a better statistical evaluation of the protection assay described in Example II. Results were shown in Figure 4 and 5. Each time-point consists of 7 animals per group. Naked DNA (100µg) constructs encoding for Rv1511. The DNA vaccine candidate was administered a total of three injections via the intramuscular route at 21 day intervals. 21 days after the last

injection the mice were aerosol challenged with *M. tuberculosis* H37Rv (200 organisms per mouse). At early (30 days) and late (60 days) time points post-challenge the bacterial load in the lung was determined as a measurement of levels of protection. Antigen Rv1511 and Rv3407 provided protection at both time points (compared to BCG) whereas, antigen Rv2520 provided protection only at the early time point. These antigens were also administered in pair-wise combinations. Splenocytes were used in ELIspot assays to determine the IFN-γ immune response to the antigens used for DNA vaccination. The increase in IFNγ production correlated well with the antigen induced in the antigen protection experiments.

These repeated protection data underline the vaccine capacity of Rv1511. The DNA vaccine encoding Rv1511 resulted in a consistent protection over all time points examined. The DNA vaccine Rv1511 induced a high protection for both time points post challenge.

Example V: Frequency of IFN-γ secretion T lymphocytes specific for Rv1511

Cytokine ELISPOT Assay. The frequency of IFNγ-secreting T lymphocytes specific for the Rv1511 H-2K^d epitope and stated in example III (Table 1) P1-P7 was determined by a slightly modified ELISPOT technique (Fensterle, J., Grode, L., Hess, J. & Kaufmann, S.H.E. (1999) *J. Immunol.* 163, 4510-4518). Briefly, 96-well nitrocellulose plates (Millititer HA; Millipore, Bedford, MA) were coated with 5 mg/ml of the anti-mouse IFNγ-mAb R4 (PharMingen) in 100 µl of carbonate buffer, pH 9.6. After an overnight incubation at 4°C, plates were washed twice with PBS and blocked for 2 h at 37°C with 100 µl of 1% BSA in PBS. Splenocytes (10^5) from vaccinated mice pulsed with 10 µg/ml P1-P7 peptides (derived from Example III) for 3 d were added in 100 µl RP10 per well. P815 cells were coated with 10 µg/ml for individual peptides (purchased from Jerini BioTools GmbH, Berlin, Germany, and dissolved in PBS) at 37°C for 1 h and then washed twice with RP10. Coated or uncoated P815 cells (10^5) were added to splenocytes in 100 µl of RP10, and after 20 h incubation at 37°C, 5% CO_2 in the presence of 30 U/ml IL-2, the plates were washed 10 times with 0.05% Tween 20 in PBS (washing buffer).

In order to detect IFNγ-spots, 0.25 µg/ml biotinylated anti-mouse IFNγ mAb XMG1.2 (PharMingen) in 100 µl washing buffer was added and incubated at 37°C for 2 h. Plates were washed 10 times in washing buffer, and incubated for 1 h at 37°C in 100 µl of a 1/20,000 dilution of alkaline phosphatase-coupled streptavidin (PharMingen). After five washes, spots of IFNγ-secreting cells were visualised by adding 50 µl of the ready-to-use substrate 5-Bromo-4-Chloro-3-Indolyl Phosphate/NitroBlue Tetrazolium (BCIP/NBT, Sigma, St. Louis, MO) dissolved in water. The reaction was stopped after 15 min at 37°C by several washes with distilled water. After drying, spots were counted under a dissecting microscope at 3-fold magnification. The frequency of peptide-specific T cells is expressed as the number of IFNγ-secreting cells per 10^5 splenocytes.

To detect Rv1511-specific MHC class II-restricted T cells, a slightly modified protocol was used. To induce specific cytokine secretion by Rv1511-specific CD4 T cells, 10^5 splenocytes per well were stimulated with 10 µg/ml heat-denatured protein lysat of H37Rv 100 µl RP10 for 3 d. J774A.1 macrophage-like cells were pulsed with 10 µg/ml heat-denatured, protein lysat of H37Rv in RP10 at 37°C for 1 h and subsequently washed twice with RP10. Coated or uncoated J774A.1 cells (10^5) were added to splenocytes in 100 µl RP10 and after 20 h incubation at 37°C, 5% CO_2 in the presence of 30 U/ml IL-2, plates were washed 10 times with 0.05% Tween 20 in PBS (washing buffer). Plates for IFNγ-detection were prepared as described above. Incubation times and the detection followed the ELISPOT protocol for MHC class I- restricted CD8 T cells.

The cytokine IFN-represents the best known marker of protection in tuberculosis. These results underline the capacity of Rv1511 to induce an acquired cellular immune response, needed to control intracellular pathogens such as M. tuberculosis. In addition, the MHC class I restricted immune response observed in this study identifies the course of protection against tuberculosis.

Example VI: Antibody titers contribute to Th1 type of immune response in Rv1511 immunized mice

Antibody response was measured by enzyme-linked immunosorbent assay (ELISA) of sera pooled from groups of vaccinated mice. Briefly, 96-well microtiter plates were coated overnight with 1µg of purified recombinant Rv1511 protein in 0.1 M carbonate buffer. Blood sera was collected from Rv1511 immunized mice. The sera were diluted and the endpoint titer was determined. Serum samples and peroxidase conjugated rabbit anti-mouse IgG (I:2500) were diluted and incubated sequentially in the plates for 2h at 37°C. For determination of antibody isotypes, antibodies specific for murine IgG1 and IgG2a were used. Sera from unimmunised mice was used as the controls.

Mice immunised with ptPA:Rv1511 exhibited enhanced antibody responses against Rv1511 as well as against an extract of M. tuberculosis. Since the Th1 arm of the cellular immune response is implicated in protective immunity against mycobacteria as shown in western-blot analysis (Fig.10), the level of IgG1 and IgG2a isotypes (Fig. 11) and consequently the ratio of IgG2a/IgG1 was determined (IgG2a/IgG1 = 3,17), to gauge the Th1/Th2 nature of the immune response. In mice, Th1 responses are characterised by strong cell-mediated and an IgG2a antibody response, whereas Th2 responses are usually associated with high-titer IgG1 and IgE antibody response.

Table 1

Seq.No.	Epitope	Position	MHC type	n-mer	Overall score	Group	Prediction Combination
3	VKRALITGI	0	HLA_Cw_0602	9	0.895942900075132	same length	PAPROC/BIMAS
	"	0	HLA_Cw_0602	9	0.895942900075132	c-term. trimmed	PAPROC/BIMAS
4	ALITGITGQ	3	HLA_A_0201	9	0.930555555555555	trimmed twice	PAPROC/SYFPEITHI
5	GITGQDGSYL	7	HLA_A_0201	10	0.931372549019608	c-term. trimmed	PAPROC/SYFPEITHI
6	GQDGSYLAEL	10	HLA_A_0201	10	0.936274509803921	same length	PAPROC/SYFPEITHI
7	QDGSYLAEL	11	HLA_Cw_0602	9	0.875073044494532	n-term. trimmed	PAPROC/BIMAS
8	LLLAKGYEV	19	HLA_A_0201	9	0.962962962962963	c-term. trimmed	PAPROC/SYFPEITHI
9	LAKGYEVHGL	21	HLA_A_0201	10	0.946078431372549	n-term. trimmed	PAPROC/SYFPEITHI
10	AKGYEVHGL	22	HLA_Cw_0602	9	0.875073044494532	n-term. trimmed	PAPROC/BIMAS
11	KGYEVHGL	23	H2_Kb	8	0.93010752688172	n-term. trimmed	PAPROC/SYFPEITHI
12	ASTFNTSRI	34	H2_Db	9	0.952380952380952	trimmed twice	PAPROC/SYFPEITHI
	"	34	H2_Db	9	0.871913580246914	trimmed twice	PAPROC/BIMAS
13	TFNTSRIDHL	36	H2_Kd	10	0.940476190476191	n-term. trimmed	FRAGPREDICT/SYFPEITHI
14	FNTSRIDHL	37	HLA_Cw_0602	9	0.879247015610652	n-term. trimmed	FRAGPREDICT/BIMAS
15	PHQPGARLF	49	HLA_B_1510	9	0.946236559139785	c-term. trimmed	PAPROC/SYFPEITHI
16	HQPGARLFL	50	HLA_Cw_0602	9	0.856290174471993	n-term. trimmed	PAPROC/BIMAS
17	QPGARLFL	51	HLA_B8	8	0.933333333333333	n-term. trimmed	PAPROC/SYFPEITHI
18	RLFLHYGDL	55	H2_Kb	9	0.871080924751195	n-term. trimmed	FRAGPREDICT/BIMAS
	"	55	HLA_A_0201	9	0.929666898451276	n-term. trimmed	FRAGPREDICT/SYFPEITHI
19	LFLHYGDL	56	H2_Kb	8	0.861737069296637	n-term. trimmed	FRAGPREDICT/BIMAS
	"	56	H2_KD	8	0.864657024126187	n-term. trimmed	FRAGPREDICT/BIMAS
	"	56	H2_Kb	8	0.945332927072265	n-term. trimmed	FRAGPREDICT/SYFPEITHI
20	TRLVTLLSTI	67	H2_Db	10	0.934343434343434	n-term. trimmed	PAPROC/SYFPEITHI
21	RLVTLLSTI	68	HLA_A_0201	9	0.949074074074074	n-term. trimmed	PAPROC/SYFPEITHI

	"	68	HLA_Cw_0602	9	0.895942900075132	n-term. trimmed	PAPROC/BIMAS
22	LLSTIEPDEV	72	HLA_A_0201	10	0.950930232252441	same length	FRAGPREDICT/SYFPEITHI
23	ASPPPQNEL	135	HLA_Cw_0602	9	0.856290174471993	c-term. trimmed	FRAGPREDICT/BIMAS
	"	135	H2_Ld	9	0.94544247878682	same length	FRAGPREDICT/SYFPEITHI
	"	135	H2_Ld	9	0.946236559139785	c-term. trimmed	FRAGPREDICT/SYFPEITHI
24	FYPRSPYGAA	146	H2_Kd	10	0.928571428571428	n-term. trimmed	FRAGPREDICT/SYFPEITHI
	"	146	H2_Kd	10	0.928532240622615	same length	FRAGPREDICT/SYFPEITHI
25	YPRSPYGAA	147	HLA_B_0702	9	0.947619047619048	trimmed twice	PAPROC/SYFPEITHI
	"	147	HLA_B_0702	9	0.947619047619048	n-term. trimmed	FRAGPREDICT/SYFPEITHI
26	PRSPYGAAK	148	HLA_B_2705	9	0.932432432432432	n-term. trimmed	PAPROC/SYFPEITHI
27	SYWATRNYR	159	HLA_A_3302	9	0.888888888888889	n-term. trimmed	PAPROC/BIMAS
28	NYREAYGL	165	H2_Kd	8	0.873521090534979	n-term. trimmed	FRAGPREDICT/BIMAS
	"	165	H2_KD	8	0.873521090534979	n-term. trimmed	FRAGPREDICT/BIMAS
29	TFVTRKITRA	190	H2_Kd	10	0.95238047249965	n-term. trimmed	FRAGPREDICT/SYFPEITHI
30	AVARIKAGI	199	HLA_Cw_0602	9	0.864638116704232	n-term. trimmed	PAPROC/BIMAS
	"	199	HLA_Cw_0602	9	0.864638116704232	trimmed twice	PAPROC/BIMAS
31	VARIKAGI	200	HLA_B8	8	0.945833333333333	n-term. trimmed	PAPROC/SYFPEITHI
	"	200	HLA_B8	8	0.945833333333333	trimmed twice	PAPROC/SYFPEITHI
32	RIKAGIQSEV	202	HLA_A_0201	10	0.926470588235008	c-term. trimmed	FRAGPREDICT/SYFPEITHI
33	IKAGIQSEV	203	HLA_Cw_0602	9	0.867768595041054	trimmed twice	FRAGPREDICT/BIMAS
	"	203	HLA_Cw_0602	9	0.867768595032501	c-term. trimmed	FRAGPREDICT/BIMAS
34	QSEVYMGNL	208	H2_Ld	9	0.940860215053763	c-term. trimmed	PAPROC/SYFPEITHI
35	VYMGNLDAV	211	H2_Db	9	0.933333333333333	n-term. trimmed	PAPROC/SYFPEITHI
36	YMGNLDAVR	212	HLA_A_3302	9	0.888888888888889	same length	PAPROC/BIMAS
	"	212	HLA_A_3302	9	0.888888888888889	c-term. trimmed	PAPROC/BIMAS
37	WGYAPEYV	222	HLA_B_5201	8	0.858585858585859	c-term. trimmed	FRAGPREDICT/BIMAS
38	YAPEYVEGM	224	H2_Db	9	0.928571428571428	trimmed twice	FRAGPREDICT/SYFPEITHI
	"	224	H2_Db	9	0.928571428571428	c-term. trimmed	FRAGPREDICT/SYFPEITHI

39	EPDDFVLAT	240	HLA_B_0702	9	0.938095238095238	trimmed twice	FRAGPREDICT/SYFPEITHI
40	VLATGRGFTV	245	HLA_A_0201	10	0.955553218369733	n-term. trimmed	FRAGPREDICT/SYFPEITHI
41	LATGRGFTV	246	HLA_B_5103	9	0.874771734620367	n-term. trimmed	FRAGPREDICT/BIMAS
42	QYVKFDQRYL	271	H2_Kd	10	0.970238095238095	n-term. trimmed	FRAGPREDICT/SYFPEITHI
	"	271	H2_Kd	10	0.970238095238095	n-term. trimmed	FRAGPREDICT/BIMAS
43	SLIGDATKA	287	HLA_A_0201	9	0.935185185185185	n-term. trimmed	FRAGPREDICT/SYFPEITHI
44	GDATKAAEL	290	HLA_Cw_0602	9	0.875048365340426	n-term. trimmed	FRAGPREDICT/BIMAS
	"	290	HLA_Cw_0602	9	0.872338351213021	trimmed twice	FRAGPREDICT/BIMAS
	"	290	HLA_Cw_0602	9	0.875073044494532	n-term. trimmed	PAPROC/BIMAS
	"	290	HLA_Cw_0602	9	0.875073044494532	trimmed twice	PAPROC/BIMAS
	"	290	HLA_Cw_0602	9	0.875073044494532	same length	PAPROC/BIMAS
	"	290	HLA_Cw_0602	9	0.875073044494532	c-term. trimmed	PAPROC/BIMAS
45	DATKAAELL	291	HLA_Cw_0602	9	0.856290174471993	n-term. trimmed	PAPROC/BIMAS
46	LLGWRASVH	298	HLA_A3	9	0.934108527131783	c-term. trimmed	FRAGPREDICT/SYFPEITHI
47	RASVHTDEL	302	HLA_Cw_0602	9	0.856041884761946	trimmed twice	FRAGPREDICT/BIMAS
	"	302	HLA_Cw_0602	9	0.856290171568337	c-term. trimmed	FRAGPREDICT/BIMAS
48	SVHTDELAR	304	HLA_A_3302	9	0.88888888737257	n-term. trimmed	FRAGPREDICT/BIMAS

References

[1] Kaufmann, S.H.E., *Nat. Med.* 2000, *6*, 955-960.
[2] Calmette, A., Guérin, C., *Ann. Inst. Pasteur* 1920, *34*, 553-360.
[3] Calmette, A., Guérin, C., Boquet, A., Negre, L., *La vaccination preventive contre la tuberculose par le "BCG"*, Masson, Paris 1927.
[4] Behr, M.A., Wilson, M.A., Gill, W.P., Salamon, H., Schoolnik, G.K., Rane, S., Small, P.M, *Science* 1999, *284*, 1520-1523.
[5] Fine, P.E., *Lancet* 1995, *346*, 1339-1345.
[6] Colditz, G.A., Brewer, T.F., Berkey, C.S., Wilson, M.E., Burdick, E., Fineberg, H.V., Mosteller, F., *JAMA* 1994, *271*, 698-702.
[7] Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., *et al.*, *Nature* 1998, *393*, 537-544.
[8] http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gmt
[9] Cole, S.T., Eiglmeier, K., Parkhill, J., James, K.D., Thomson, N.R., Wheeler, P.R., *et al.*, *Nature* 2001, *409*, 1007-1011.
[10] http://www.tigr.org/tdb/CMR/gmt/htmls/SplashPage.html
[11] Heifets, L.B., Good, R.C., in: Bloom, B.R. (Ed.), *Tuberculosis: Pathogenesis, Protection and Control*, American Society for Microbiology Press, Washington DC 1994, pp. 85-110.
[12] Mahairas, G. G., Sabo, P. J., Hickey, M. J., Singh, D. C., Stover, C. K., *J. Bacteriol.* 1996, *178*, 1274-1282.
[13] Philipp, W.J., Nair, S., Guglielmi, G., Lagranderie, M., Gicquel, B., Cole, S.T., *Microbiology* 1996, *142*, 3135-3145.
[14] Brosch, R., Gordon, S.V., Billault, A., Garnier, T., Eiglmeier, K., Soravito, C., Barrell, B.G., Cole, S.T., *Infect. Immun.* 1998, *66*, 2221-2229.
[15] GORDON, S.V., BROSCH, R., BILLAULT, A., GARNIER, T., EIGLMEIER, K., COLE, S.T., *MOL. MICROBIOL.* 1999, *32*, 643-655.
[16] Ahmad, S., Amoudy, H.A., Thole, J.E., Young, D.B., Mustafa, A.S., *Scand. J. Immunol.* 1999, *49*, 515-522.
[17] Jungblut, P., Thiede, B., Zimny-Arndt, U., Müller, E.-C., Scheler, C., Wittmann-Liebold, B., Otto, A., *Electrophoresis* 1996, *17*, 839-847.
[18] Britton, W.J., Hellqvist, L., Ivanyi, J., Basten, A., *Scand. J. Immunol.* 1987, *26*, 149-159.
[19] Gulle, H., Schoel, B., Kaufmann, S.H., *J. Immunol. Methods* 1990, *133*, 253-261.
[20] Daugelat, S., Gulle, H., Schoel, B., Kaufmann, S.H.E., *J. Infect. Dis.* 1992, *166*, 186-190.
[21] Wallis, R.S., Paranjape, R., Phillips, M., *Infect. Immun.* 1993, *61*, 627-632.
[22] Lee, B.Y., Horwitz, M.A., *J. Clin. Invest.* 1995, *96*, 245-249.
[23] Urquhart, B.L., Atsalos, T.E., Roach, D., Basseal, D. J., Bjellqvist, B., Britton, W.L., Humphery-Smith, I., *Electrophoresis* 1997, 18, 1384-1392.
[24] Sonnenberg, M.G., Belisle, J.T., *Infect. Immun.* 1997, 65, 4515-4524.
[25] Weldingh, K., Rosenkrands, I., Jacobsen, S., Rasmussen, P.B., Elhay, M.J., Andersen, P., *Infect. Immun.* 1998, *66*, 3492-3500.

[26] Jungblut et al., Mol. Microbiol. 1999, *33*, 1103-1117.
[27] Mollenkopf, H.J., Jungblut, P.R., Raupach, B., Mattow, J., Lamer, S., Zimny-Arndt, U., Schaible, U.E., Kaufmann S.H.E., *Electrophoresis* 1999, *20*, 2172-2180.
[28] Rosenkrands, I., Weldingh, K., Jacobsen, S., Hansen, C.V., Florio, W., Gianetri, I., Andersen, P., *Electrophoresis* 2000, *21*, 935-948.
[29] http://www.mpiib-berlin.mpg.de/2D-PAGE/
[30] http://www.who.int/vaccines-diseases/safety/infobank/bcg.htm
[31] Klose, J., Kobalz, U., *Electrophoresis* 1995, *16*, 1034-1059.
[32] Jungblut P.R., Seifert R., *J. Biochem. Biophys. Methods* 1990, *21*, 47-58.
[33] Scheler, C., Lamer, S., Pan, Z., Li, X.P., Salnikow, J., Jungblut, P., *Electrophoresis* 1998, *19*, 918-927.
[34] Müller, E.C., Schümann, M., Rickers, A., Bommert, K., Wittmann-Liebold, B., Otto, A., *Electrophoresis* 1999, *20*, 320-330.
[35] Otto A., Thiede B., Müller E.C., Scheler C., Wittmann-Liebold B., Jungblut P., – *Electrophoresis* 1996, *17*, 1643-1650.
[36] Henzel, W. J., Billeci, T. M., Stults, J. T., Wong, S. C., Grimley, C., Watanabe, C., *Proc. Natl. Acad. Sci. USA* 1993, *90*, 5011-5015.
[37] James, P., Quadroni, M., Carafoli, E., Gonnet, G., *Biochem. Biophys. Res. Commun.* 1993, *195*, 58-64.
[38] Mann, M., Hojrup, P., Roepstorff, P., *Biol. Mass Spectrom.* 1993, *22*, 338-345.
[39] Pappin, D. J. C., Hojrup, P., Bleasby, A. J., *Curr. Biol.* 1993, 3, 327-332.
[40] http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Protein
[41] http://prospector.ucsf.edu/ucsfhtml3.2/msfit.htm
[42] Spengler, B., Kirsch, D., Kaufmann, R., Jaeger, E., *Rapid Commun. Mass Spectrom.* 1992, *6*, 105-108.
[43] Mann, M., Wilm, M., *Anal. Chem.* 1994, *66*, 4390-4399.
[44] Wilm, M., Mann, M., *Anal. Chem.* 1996, *68*, 1-8.
[45] Sreevatsan, S., Pan, X., Stockbauer, K.E., Connell, N.D., Kreiswirth, B.N., Whittam, T.S., Musser, J.M., *Proc. Natl. Acad. Sci. USA* 1997, *94*, 9869-9874.
[46] H.-G. Holzhütter, C. Frömmel, and P.-M. Kloetzel (1999). A Theoretical Approach Towards the Identification of Cleavage-Determining Amino Acid Motifs of the 20S Proteasome. J. Mol. Biol. 286, 1251-1265
[47] C. Kuttler, A.K. Nussbaum, T.P. Dick, H.-G. Rammensee, H. Schild, K.P. Hadeler (2000). An algorithm for the prediction of proteasomal cleavages, J. Mol. Biol. 298, 417-429
[48] K. C. Parker, M. A. Bednaree, and J. E. Coligan (1994). Scheme for ranking potential HLA-A2 binding peptides based on independent binding of individual peptide side-chains. J. Immunol. 152:163
[49] H.-G. Rammensee, J. Bachmann, N.N. Emmerich, O.A. Bachor, and S. Stevanovic (1999). SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics 50, 213-219
[50] K. C. Parker, M. A. Bednarek, L. K. Hull, U. Utz, B. Cunningham, H. J. Zweerink, W. E. Biddison and J. E. Coligan (1992), J. Immunol. 149, 3580-3587
[52] A. K. Nussbaum, C. Kuttler, K. P. Hadeler, H. G. Rammensee and H. Schild (2001), Immunogenetics 53, 87-94
[53] H. G. Holzhutter and P. M. Kloetzel (2000), Biophys. J. 79, 1196-205

[54] Mustafa, A.S., Amoudy, H.A., Wiker, H.G., Abal, A.T., Ravn, P., Oftung, F., Andersen, P., *Scand. J. Immunol.* 1998, *48*, 535-543.

[55] Rosenkrands, I., Rasmussen, P.B., Carnio, M., Jacobsen, S., Theisen, M., Andersen, P., *Infect. Immun.* 1998, *66*, 2728-2735.

[56] Ulrichs, T., Anding, P., Porcelli, S., Kaufmann, S.H.E., Munk, M.E., *Infect. Immun.* 2000, *68*, 6073-6076.

[57] Kamath, A.T., Feng, C.G., Macdonald, M., Briscoe, H., Britton, W.J., *Infect. Immun.* 1999, *67*, 1702-1707.

[58] Blower, S. M., P. M. Small, and P. C. Hopewell. 1996. Control strategies for tuberculosis epidemics: new models for old problems. Science. 273(5274):497-500.

[59] Davis, H. L. 1997. Plasmid DNA expression systems for the purpose of immunization. Curr Opin Biotechnol. 8(5):635-46.

[60] Davis, H. L., and R. G. Whalen. 1995. DNA-based immunization. Mol Cell Biol Hum Dis Ser. 5:368-87.

[61] Denis, O., A. Tanghe, K. Palfliet, F. Jurion, T. P. van den Berg, A. Vanonckelen, J. Ooms, E. Saman, J. B. Ulmer, J. Content, and K. Huygen. 1998. Vaccination with plasmid DNA encoding mycobacterial antigen 85A stimulates a CD4+ and CD8+ T-cell epitopic repertoire broader than that stimulated by Mycobacterium tuberculosis H37Rv infection. Infect Immun. 66(4):1527-33.

[62] Donnelly, J. J., J. B. Ulmer, J. W. Shiver, and M. A. Liu. 1997. DNA vaccines. Annu Rev Immunol. 15:617-48.

[63] Feltquate, D. M. 1998. DNA vaccines: vector design, delivery, and antigen presentation. J Cell Biochem Suppl. 30-31:304-11.

[64] Huygen, K., J. Content, O. Denis, D. L. Montgomery, A. M. Yawman, R. R. Deck, C. M. DeWitt, I. M. Orme, S. Baldwin, D. S. C, A. Drowart, E. Lozes, P. Vandenbussche, J. P. Van Vooren, M. A. Liu, and J. B. Ulmer. 1996. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine [see comments]. Nat Med. 2(8):893-8.

[65] Kamath, A. T., C. G. Feng, M. Macdonald, H. Briscoe, and W. J. Britton. 1999. Differential protective efficacy of DNA vaccines expressing secreted proteins of Mycobacterium tuberculosis. Infection & Immunity. 67(4):1702-1707.

[66] Lozes, E., K. Huygen, J. Content, O. Denis, D. L. Montgomery, A. M. Yawman, P. Vandenbussche, J. P. Van Vooren, A. Drowart, J. B. Ulmer, and M. A. Liu. 1997. Immunogenicity and efficacy of a tuberculosis DNA vaccine encoding the components of the secreted antigen 85 complex. Vaccine. 15(8):830-3.

[67] Montgomery, D. L., K. Huygen, A. M. Yawman, R. R. Deck, C. M. Dewitt, J. Content, M. A. Liu, and J. B. Ulmer. 1997. Induction of humoral and cellular immune responses by vaccination with M. tuberculosis antigen 85 DNA. Cell Mol Biol (Noisy-Le-Grand). 43(3):285-92.

[68] Ramsay, A. J., K. H. Leong, and I. A. Ramshaw. 1997. DNA vaccination against virus infection and enhancement of antiviral immunity following consecutive immunization with DNA and viral vectors. Immunol Cell Biol. 75(4):382-8.

[69] Schirmbeck, R., W. Bohm, and J. Reimann. 1996. DNA vaccination primes MHC class I-restricted, simian virus 40 large tumor antigen-specific CTL in H-2d mice that reject syngeneic tumors. J Immunol. 157(8):3550-8.

[70] Strugnell, R. A., D. Drew, J. Mercieca, S. DiNatale, N. Firez, S. J. Dunstan, C. P. Simmons, and J. Vadolas. 1997. DNA vaccines for bacterial infections. Immunol Cell Biol. 75(4):364-9.

[71] Tascon, R. E., M. J. Colston, S. Ragno, E. Stavropoulos, D. Gregory, and D. B. Lowrie. 1996. Vaccination against tuberculosis by DNA injection. Nat Med. 2(8):888-92.

[72] Torres, C. A., A. Iwasaki, B. H. Barber, and H. L. Robinson. 1997. Differential dependence on target site tissue for gene gun and intramuscular DNA immunizations. J Immunol. 158(10):4529-32.

[73] Ulmer, J. B., M. A. Liu, D. L. Montgomery, A. M. Yawman, R. R. Deck, C. M. DeWitt, J. Content, and K. Huygen. 1997. Expression and immunogenicity of Mycobacterium tuberculosis antigen 85 by DNA vaccination. Vaccine. 15(8):792-4.

Claims

1. A pharmaceutical composition comprising at least one polypeptide selected from the group consisting of
   (a) a polypeptide encoded by a polynucleotide comprising a nucleic acid sequence as shown in SEQ ID NO: 1;
   (b) a polypeptide comprising an amino acid sequence as shown in SEQ ID NO: 2;
   (c) a polypeptide which is or which comprises a functional domain, an antigenic fragment and/or a fragment capable of eliciting and/or triggering an immune response in a mammal of the polypeptide of (a) or (b);
   (d) a polypeptide which is encoded by a polynucleotide which is at least 80% identical to the polynucleotide as defined in (a) and which is capable of eliciting and/or triggering an immune response in a mammal; and
   (e) a polypeptide which is encoded a polynucleotide which hybridizes under stringent conditions with the polynucleotide as defined in (a) or (d) and is capable of eliciting and/or triggering an immune response in a mammal.

2. The pharmaceutical composition of claim 1 wherein said polypeptide is part of a fusion protein.

3. The pharmaceutical composition of claim 2, wherein said fusion protein comprises an immunostimulatory molecule.

4. The pharmaceutical composition of claim 2 or 3, wherein said fusion protein comprises a molecule which is capable of optimizing antigen processing or comprises a peptide or polypeptide capable of eliciting and/or triggering an immune response in a mammal.

5. The pharmaceutical composition of any one of claims 1 to 4, wherein said functional domain, said antigenic fragment and/or said fragment capable of eliciting and/or triggering an immune response in a mammal is selected from the group consisting of the amino acid sequences as depicted in any one of SEQ ID NOs: 3 to 48.

6. The pharmaceutical composition of claim 5, wherein said functional domain, said antigenic fragment and/or said fragment capable of eliciting and/or triggering an immune response in a mammal is the peptide shown in SEQ ID NO: 13, 20, 23, 24, 29, 35 or 42.

7. A pharmaceutical composition comprising at least one polynucleotide encoding the polypeptide or the fusion protein as defined in any one of claim 1 to 6.

8. The pharmaceutical composition of claim 7, wherein said polynucleotide is comprised in a vector.

9. A pharmaceutical composition comprising a host cell which comprises the polynucleotide as defined in claim 7 or the vector as defined in claim 8.

10. A pharmaceutical composition comprising an antibody or fragment or a derivative thereof directed against a polypeptide as defined in any one of claims 1 to 6.

11. A pharmaceutical composition comprising a recombinant bacterial host cell of an avirulent strain or a vaccine strain which comprises at least one polynucleotide as defined in claim 7 or a vector of claim 8..

12. The pharmaceutical composition of claim 10, wherein said recombinant cell comprises at least one further nucleic acid molecule/polynucleotide encoding a peptide or polypeptide capable of eliciting and/or trigger an immune response in a mammal.

13. The pharmaceutical composition of claim 4 or 11, wherein said peptide or polypeptide capable of eliciting and/or trigger an immune response is selected from the group consisting of auto-antigens, tumor-antigens, virus-antigens, parasite-antigens, bacterial-antigens and/or immunogenic fragments thereof.

14. The pharmaceutical composition of any one of claims 10 to 13, wherein said cell is capable of expressing at least one recombinant nucleic acid molecule.

15. The pharmaceutical composition of any one of claims 1 to 14, which is a vaccine.

16. The pharmaceutical composition of any one of claims 10 to 15 which is a living vaccine suitable for administration to a mucosal surface via the parenteral route

17. A recombinant bacterial host cell of an avirulent strain or a vaccine strain which comprises at least one polynucleotide as defined in claim 7.

18. The recombinant cell of claim 17, which comprises at least one further nucleic acid molecule encoding a peptide or polypeptide capable of eliciting and/or trigger an immune response in a mammal.

19. The cell of claim 17 or 18, wherein the peptide or polypeptide capable of eliciting and/or trigger an immune response is selected from the group consisting of autoantigens, tumor-antigens, virus-antigens, parasite-antigens, bacterial-antigens and/or immunogenic fragments thereof.

20. The cell according to any one of claims 17 to 19, which is capable of expressing said polynucleotide and said at least one further recombinant nucleic acid molecule.

21. A fusion protein as defined in any one of claims 2 to 6.

22. A polynucleotide encoding for the fusion protein of claim 21.

23. A method for the production of a vaccine against a virulent strain of the genus Mycobacterium comprising the step of combining a polypeptide as defined in any one of claims 1 to 6 with a pharmaceutically acceptable carrier.

24. The method of claim 23, wherein said polypeptide is recombinantly produced.

25. The method of claim 23 or 24 which further comprises the recombinant expression of a polynucleotide as defined in claim 6 for recombinantly producing said polypeptide.

26. A method for the production of a vaccine against a virulent strain of the genus Mycobacterium comprising the step of combining a peptide as shown in any one of SEQ ID NOs: 3 to 48 with a pharmaceutically acceptable carrier.

27. A method for the production of a vaccine against a virulent strain of the genus Mycobacterium comprising the step of combining a polynucleotide as defined in claim 7 or a vector as defined in claim 8 with a biologically acceptable carrier, wherein said polynucleotide or said polynucleotid comprised in said vector is placed under the control of an expression control sequence.

28. A method for the production of an antibody or a fragment thereof directed against a polypeptide as defined in claim 1 comprising the steps of

(a) administering to an non-human animal a peptide as shown in any one SEQ ID NOs. 3 to 48 or a nucleic acid molecule encoding a peptide as shown in any one SEQ ID NOs. 3 to 48;

(b) eliciting an immune response in said non-human animal; and

(c) isolating antibodies or fragments thereof generated in said non-human animal.

29. A method for the production of an antibody or fragments thereof directed against a polypeptide as defined in claim 1 comprising the steps of

(a) administering to an non-human animal a peptide as shown in any one SEQ ID NOs. 3 to 48 or a nucleic acid molecule encoding a peptide as shown in any one SEQ ID NOs. 3 to 48;

(b) eliciting an immune response in said non-human animal;

(c) isolating from said non-human animal a producing said antibody;

(d) fusing said isolated cell to an immortalized cell;

(e) culturing the resulting hybridoma cell; and

(f) isolating from the culture medium the antibody secreted.

30 The method of claim 29 further comprising the modification of the isolated antibody and/or the production of a modified antibody molecule or an antibody derivative.

31. Use of a polynucleotide as defined in 7 or a vector as defined in claim 8 for the preparation of a vaccine for vaccination against a virulent strain of the genus Mycobacterium or against a mycobacterium-induced disease.

32. Use of a peptide as depicted in any one of SEQ ID NOs: 3 to 48 for the preparation of a vaccine for the vaccination against a virulent strain of the genus Mycobacterium or against a mycobacterium-induced disease.

33. The use of claim 32, wherein said peptide is selected from the group consisting of SEQ ID NO: 13, 20, 23, 24, 29, 35 or 42.

34. The use of any one of claims 31 to 33, wherein said mycobacterium-induced disease is selected from the group consisting of tuberculosis, tropical skin ulcer, ulceration, absess, granulomatous (skin) disease, pulmonary disease, lymphadenitis, and cutaneous and disseminated disease.

35. An isolated peptide selected from the group consisting of a peptide as depicted in any one of SEQ ID NOs: 3 to 48 or an isolated peptide which is at least 80% homologous to a peptide as shown in SEQ ID NOs: 3 to 48.

36. A nucleic acid molecule encoding a peptide of claim 35.

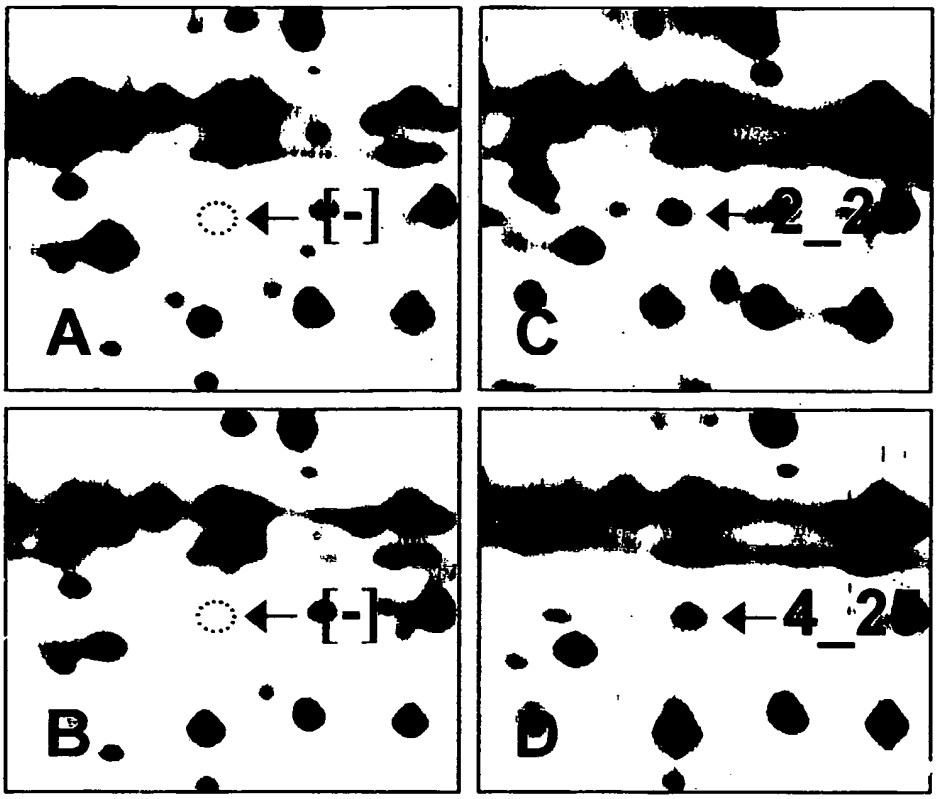


Fig. 1. Sectors from two-dimensional electrophoretic patterns of total cell proteins of attenuated (**A**: *M. bovis* BCG Chicago; **B**: *M. bovis* BCG Copenhagen) and virulent (**C**: *M. tuberculosis* H37Rv; **D**: *M. tuberculosis* Erdman) mycobacterial strains. The spots 2_25 of *M. tuberculosis* H37Rv and 4_25 of *M. tuberculosis* Erdman had no counterparts in the protein patterns of the attenuated *M. bovis* BCG strains and were identified as probable GDP-D-mannose dehydratase (encoded by the ORF Rv1511) of *M. tuberculosis* H37Rv.

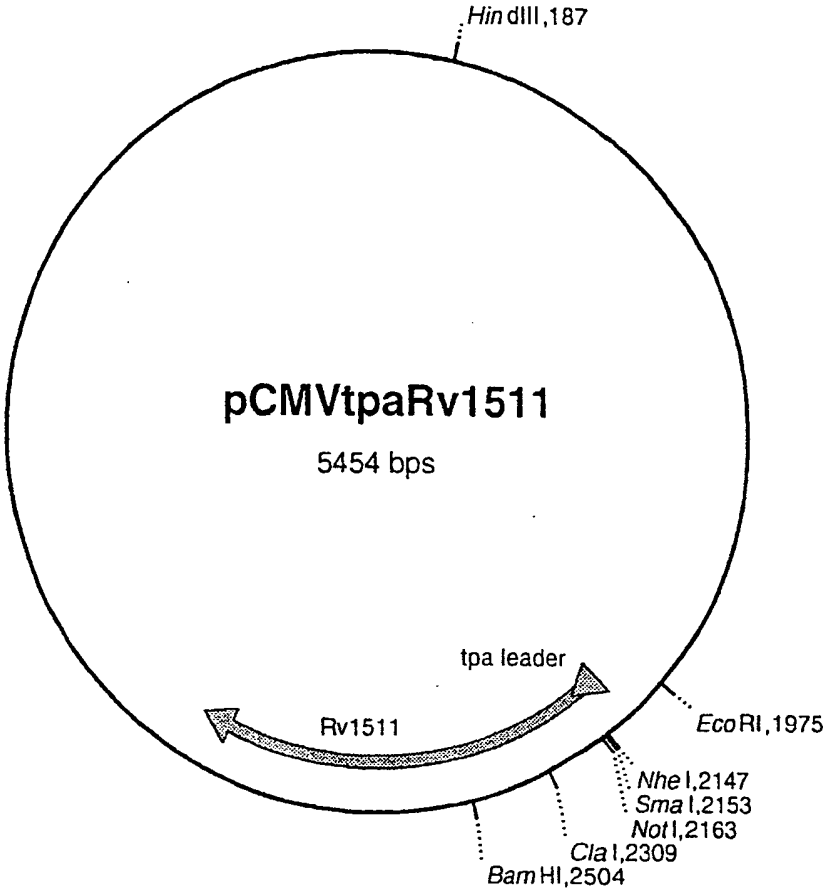

HindIII,187
pCMVtpaRv1511
5454 bps
tpa leader
Rv1511
EcoRI,1975
NheI,2147
SmaI,2153
NotI,2163
ClaI,2309
BamHI,2504

Fig. 2

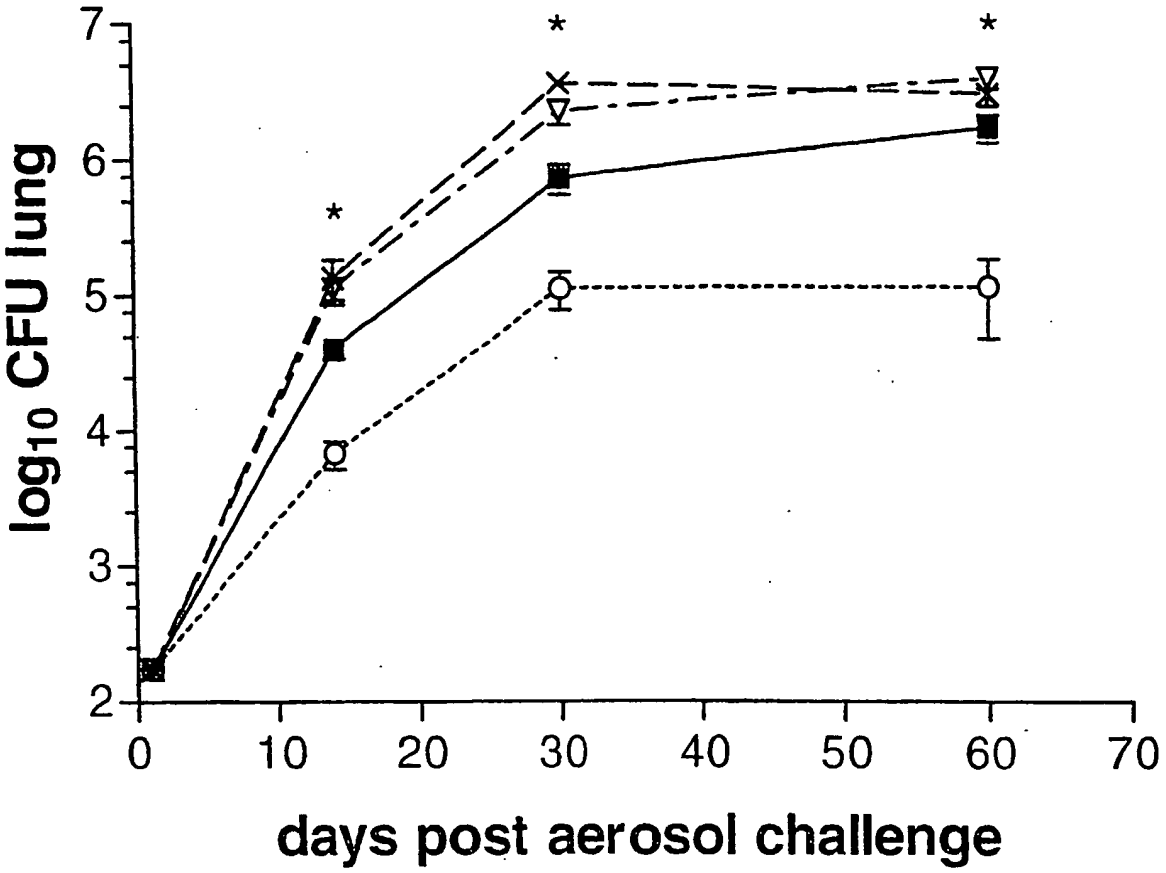
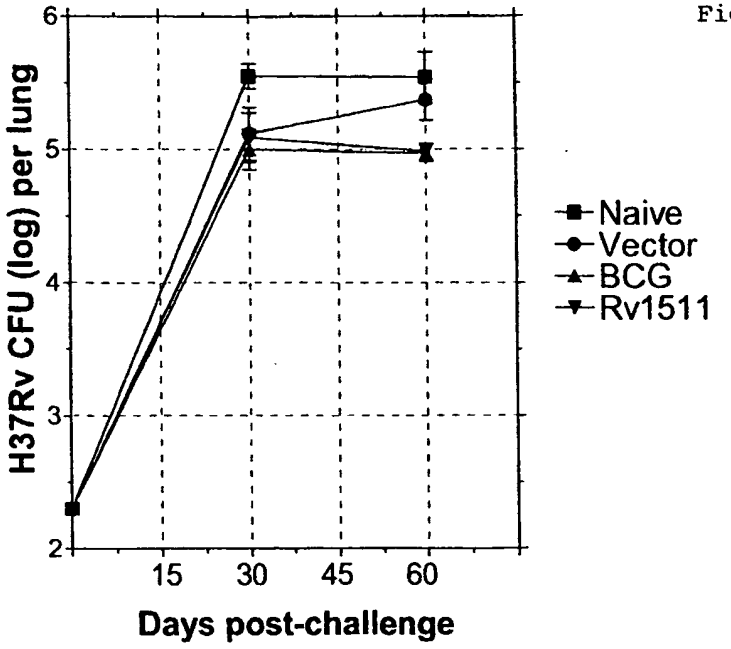

log10 CFU lung
7
6
5
4
3
2
0
10
20
30
40
50
60
70
days post aerosol challenge
*
*
*

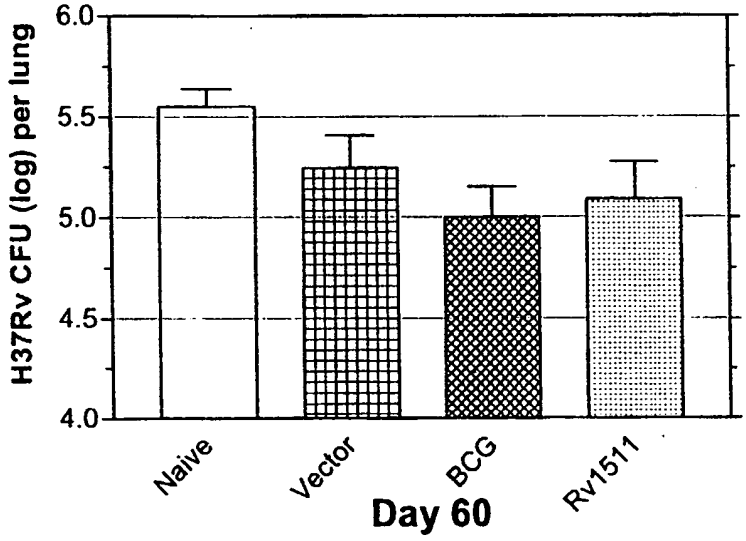
Fig. 3

Fig. 4

A)


H37Rv CFU (log) per lung
6
5
4
3
2
15
30
45
60
Days post-challenge
Naive
Vector
BCG
Rv1511

B)


Day 30
H37Rv CFU (log) per lung
6.0
5.5
5.0
4.5
4.0
Naive
Vector
BCG
Rv1511

C)

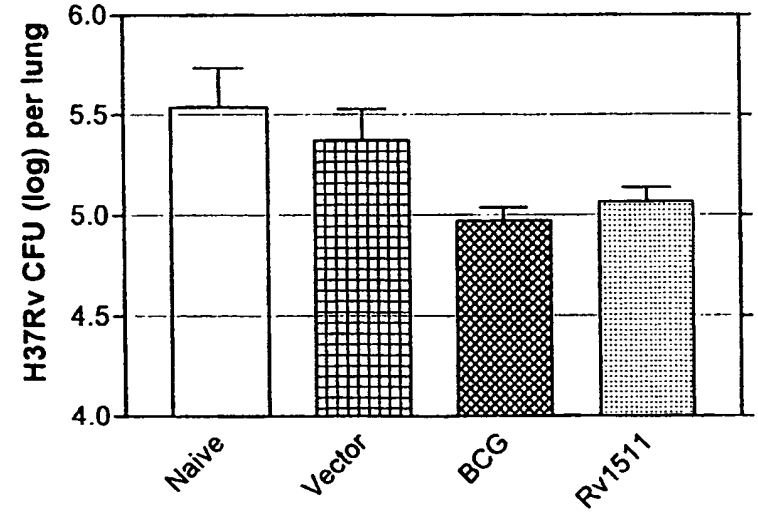

Day 60
H37Rv CFU (log) per lung
6.0
5.5
5.0
4.5
4.0
Naive
Vector
BCG
Rv1511

Fig. 5

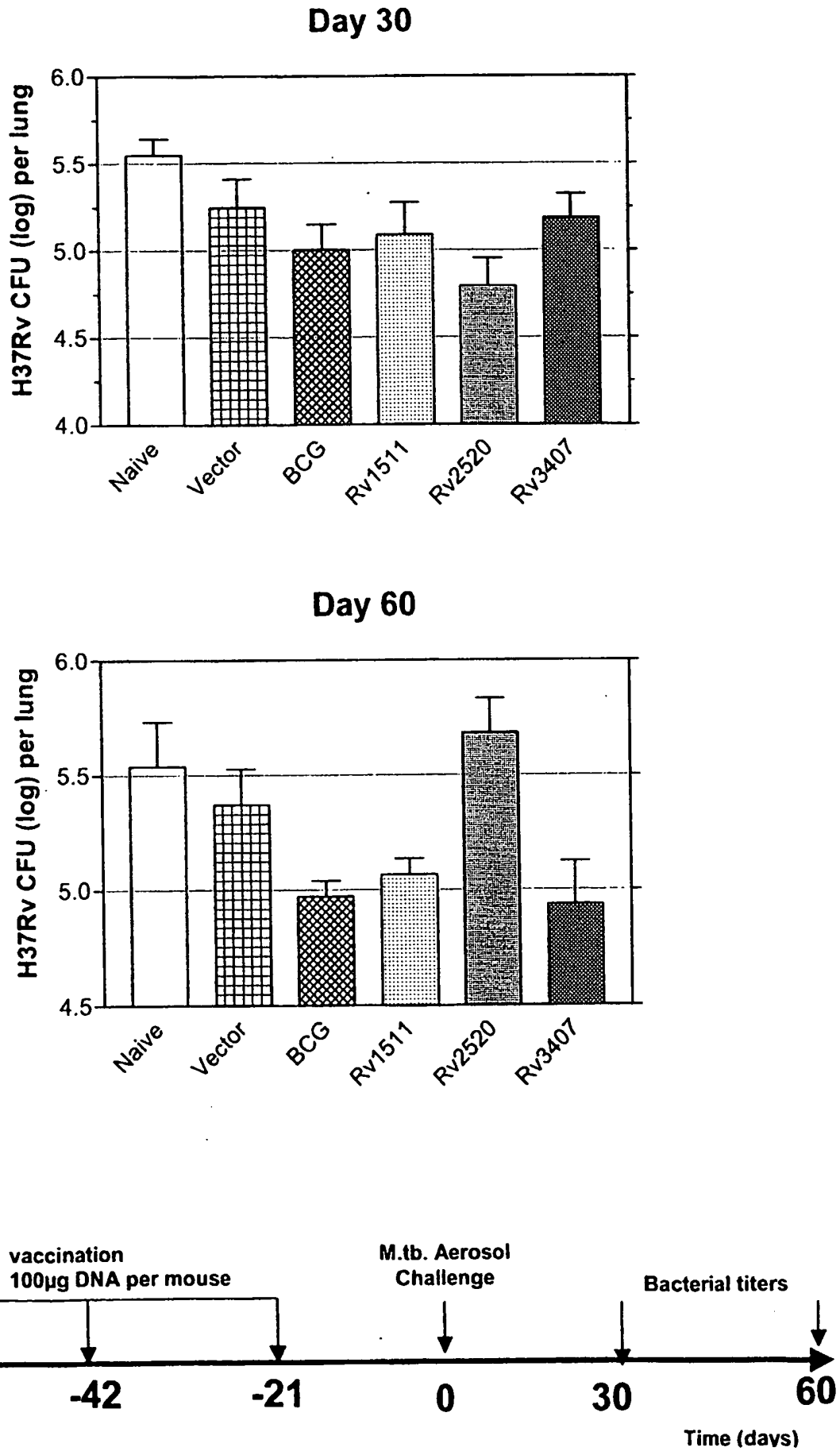
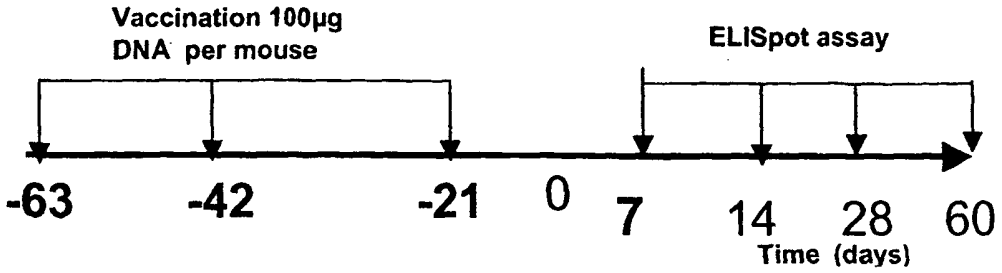

Day 30
H37Rv CFU (log) per lung
6.0
5.5
5.0
4.5
4.0
Naive
Vector
BCG
Rv1511
Rv2520
Rv3407
Day 60
H37Rv CFU (log) per lung
6.0
5.5
5.0
4.5
Naive
Vector
BCG
Rv1511
Rv2520
Rv3407
vaccination
100µg DNA per mouse
M.tb. Aerosol
Challenge
Bacterial titers
-63
-42
-21
0
30
60
Time (days)

Fig.6


Vaccination 100µg
DNA per mouse
ELISpot assay
-63
-42
-21
0
7
14
28
60
Time (days)

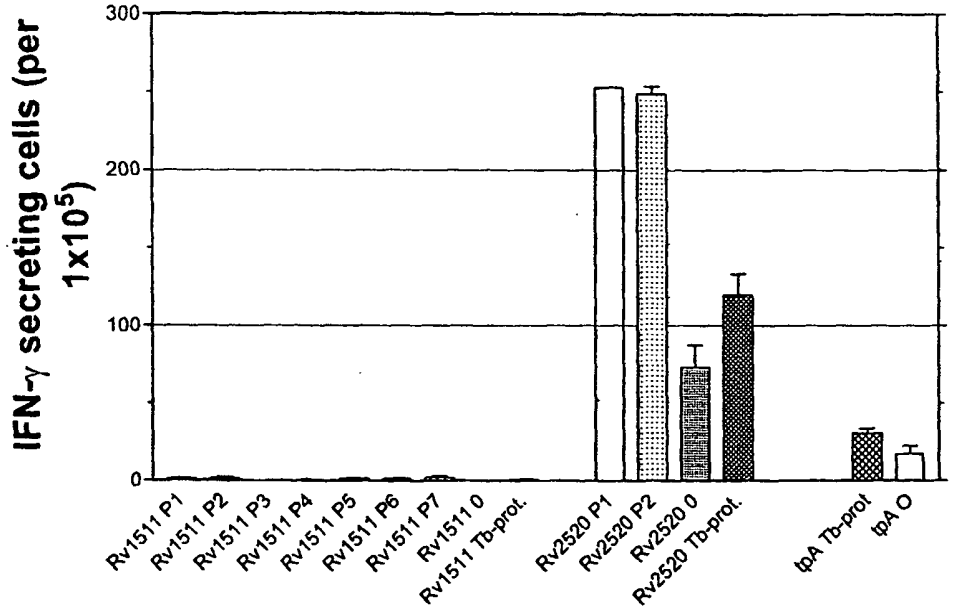
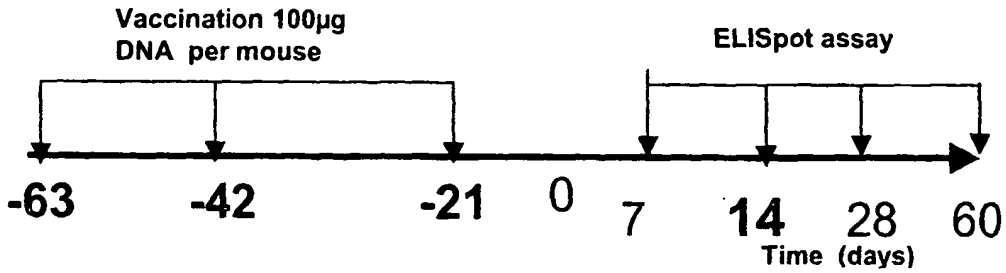

IFN-γ secreting cells (per 1x10^5)
300
200
100
0
Rv1511 P1
Rv1511 P2
Rv1511 P3
Rv1511 P4
Rv1511 P5
Rv1511 P6
Rv1511 P7
Rv1511 0
Rv1511 Tb-prot.
Rv2520 P1
Rv2520 P2
Rv2520 0
Rv2520 Tb-prot.
tpA Tb-prot
tpA O

Fig.7


Vaccination 100µg
DNA per mouse
ELISpot assay
-63
-42
-21
0
7
14
28
60
Time (days)

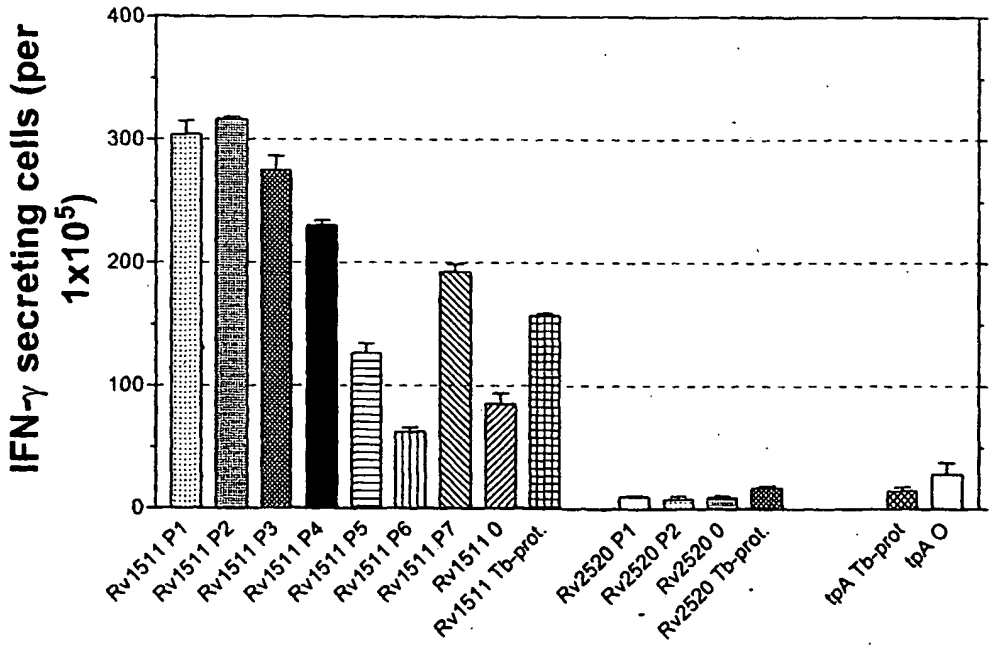
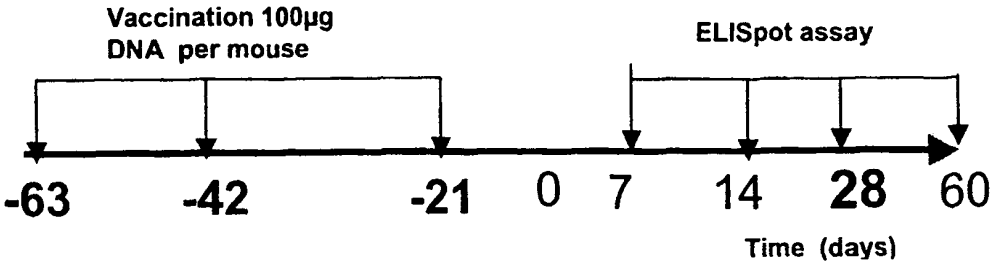

IFN-γ secreting cells (per 1x10^5)
400
300
200
100
0
Rv1511 P1
Rv1511 P2
Rv1511 P3
Rv1511 P4
Rv1511 P5
Rv1511 P6
Rv1511 P7
Rv1511 0
Rv1511 Tb-prot.
Rv2520 P1
Rv2520 P2
Rv2520 0
Rv2520 Tb-prot.
tpA Tb-prot
tpA O

Fig.8


Vaccination 100µg
DNA per mouse
ELISpot assay
-63
-42
-21
0
7
14
28
60
Time (days)

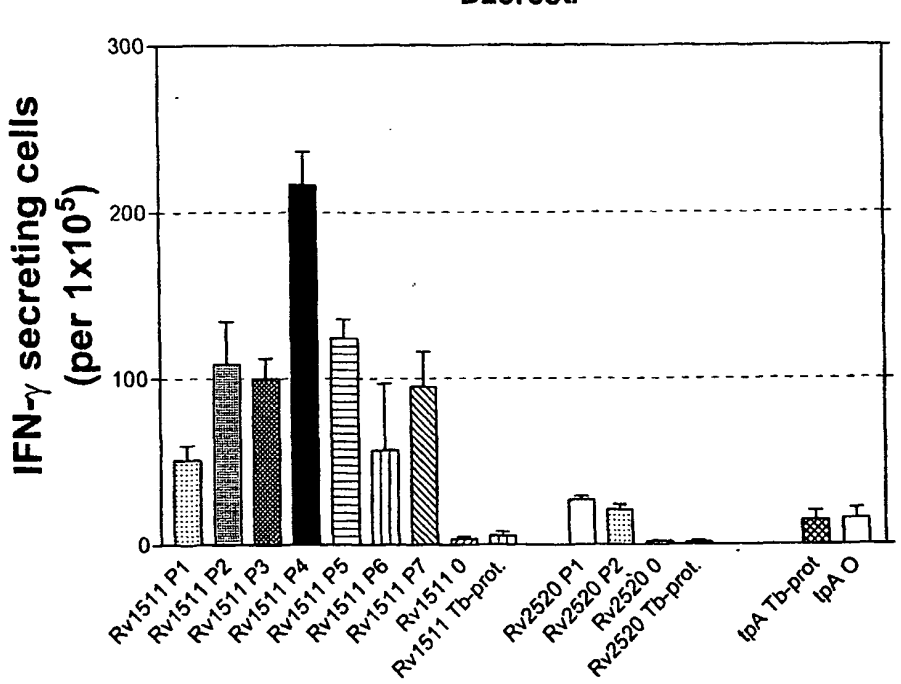
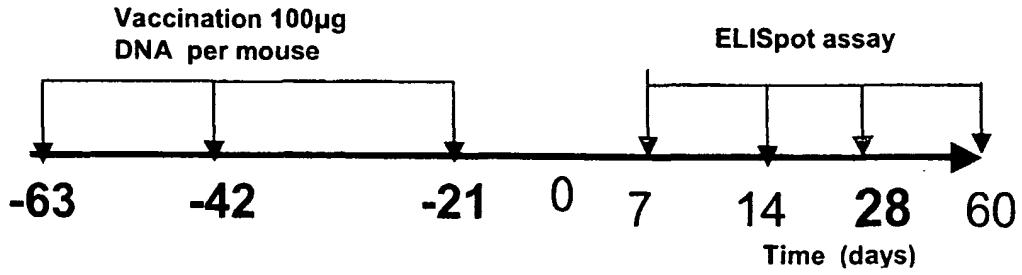

D28rest.
IFN-γ secreting cells
(per 1x10^5)
300
200
100
0
Rv1511 P1
Rv1511 P2
Rv1511 P3
Rv1511 P4
Rv1511 P5
Rv1511 P6
Rv1511 P7
Rv1511 0
Rv1511 Tb-prot.
Rv2520 P1
Rv2520 P2
Rv2520 0
Rv2520 Tb-prot.
tpA Tb-prot
tpA O

Fig.9


Vaccination 100µg
DNA per mouse
ELISpot assay
-63
-42
-21
0
7
14
28
60
Time (days)

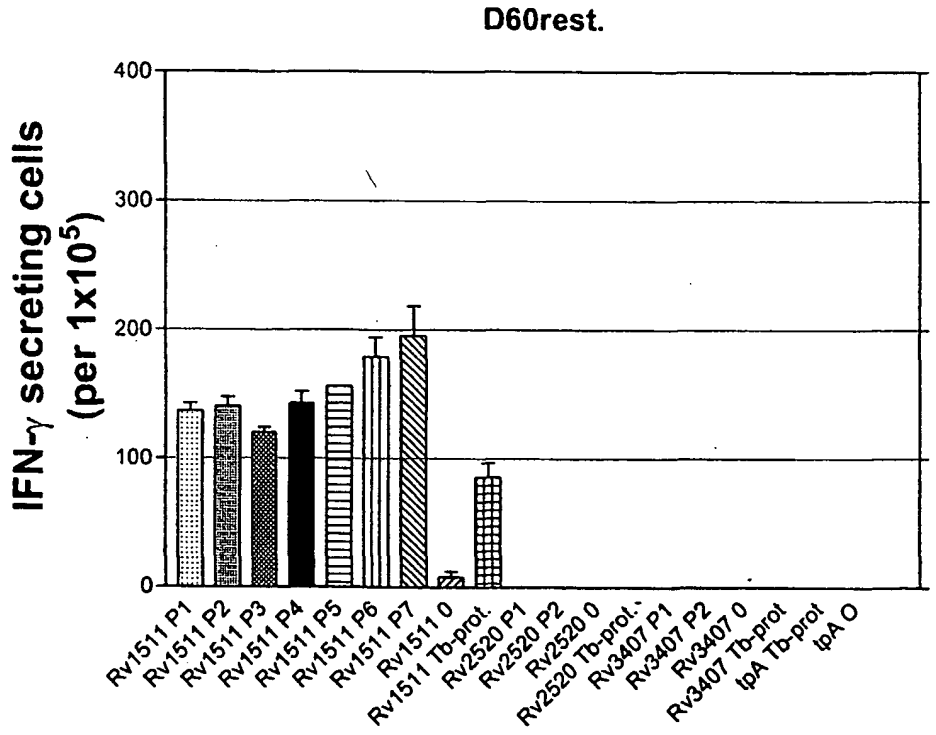

D60rest.
IFN-γ secreting cells
(per 1x10^5)
400
300
200
100
0
Rv1511 P1
Rv1511 P2
Rv1511 P3
Rv1511 P4
Rv1511 P5
Rv1511 P6
Rv1511 P7
Rv1511 0
Rv1511 Tb-prot.
Rv2520 P1
Rv2520 P2
Rv2520 0
Rv2520 Tb-prot.
Rv3407 P1
Rv3407 P2
Rv3407 0
Rv3407 Tb-prot
tpA Tb-prot
tpA O

Fig. 10

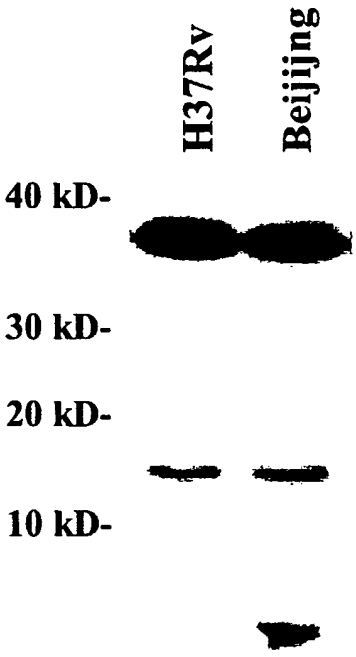
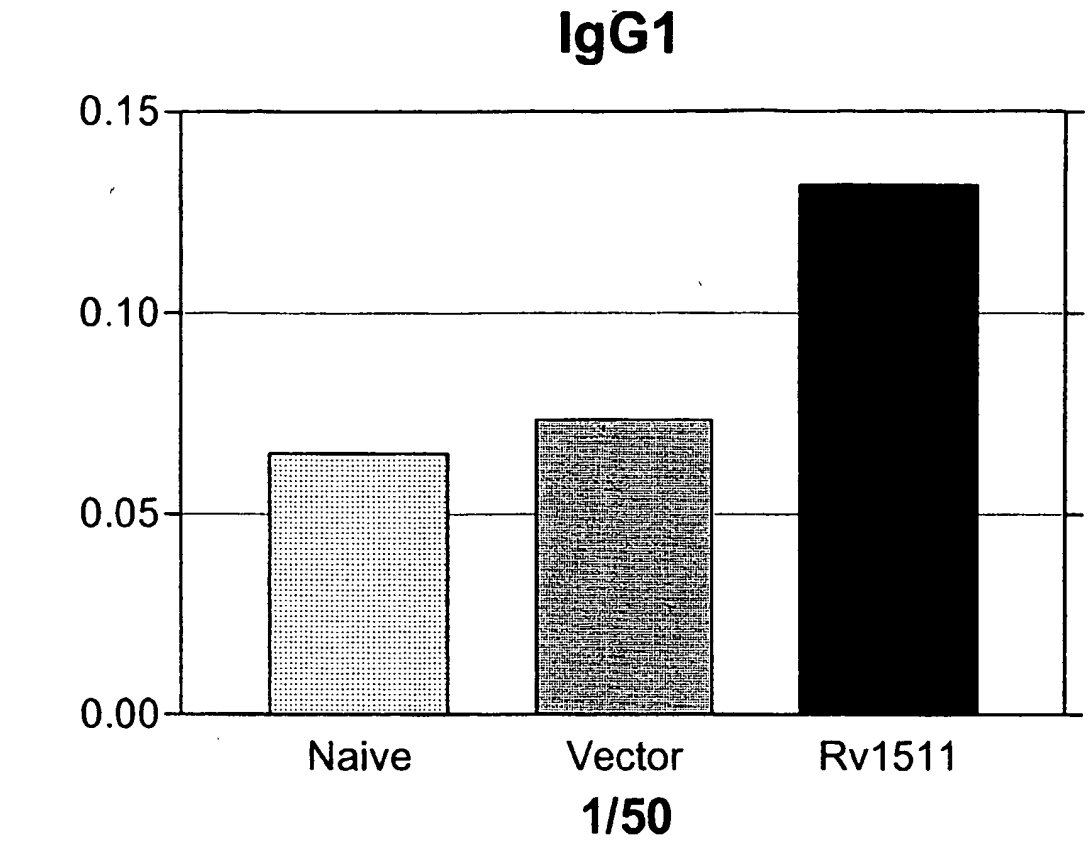
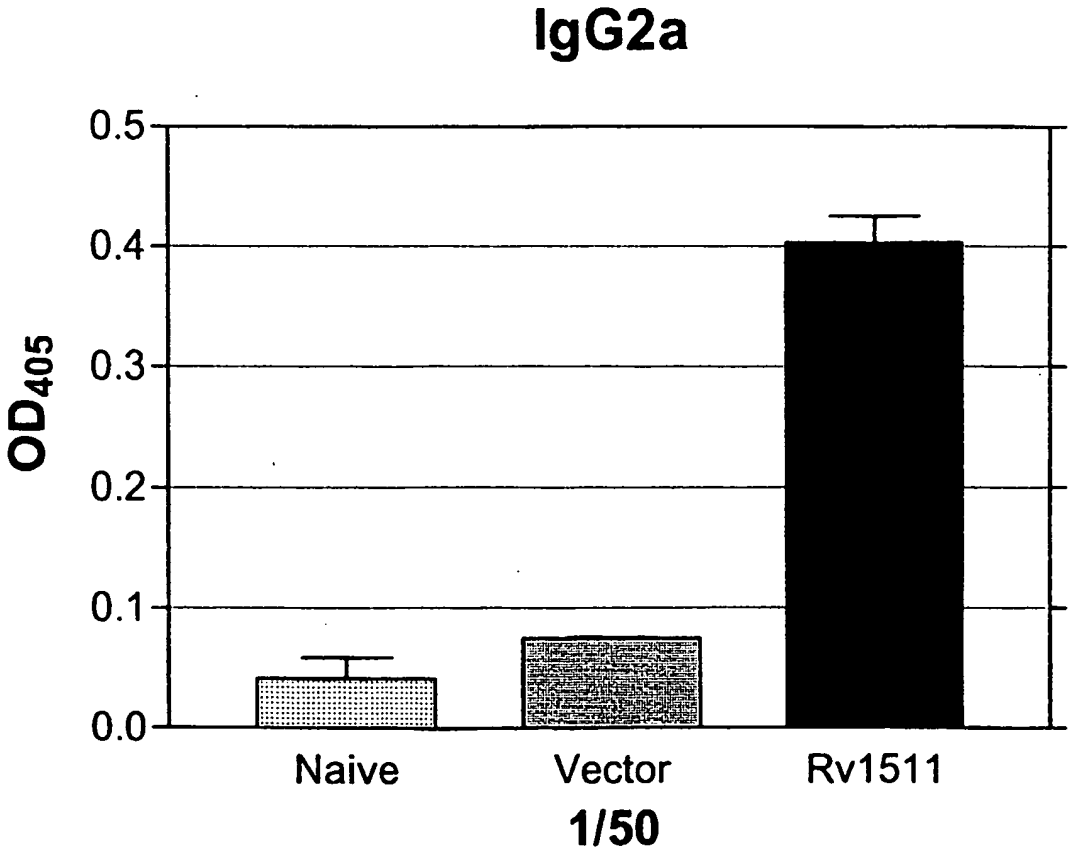


Fig. 11


IgG1
0.15
0.10
0.05
0.00
Naive
Vector
Rv1511
1/50


IgG2a
OD405
0.5
0.4
0.3
0.2
0.1
0.0
Naive
Vector
Rv1511
1/50

SEQUENCE LISTING

<110> Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.

<120> Vaccine against mycobacterial-induced diseases

<130> F 2158 PCT

<150> 01 12 0194.4
<151> 2001-08-22

<160> 50

<170> PatentIn version 3.1

<210> 1
<211> 1023
<212> DNA
<213> mycobacterium tuberculosis

<400> 1
gtgaagcgag cgctcatcac cggaatcacc ggccaggacg gctcgtatct cgccgaactg 60

ctgctggcca aggggtatga ggttcacggg ctcatccggc gcgcttcgac gttcaacacc 120

tcgcggatcg atcacctcta cgtcgacccg caccaaccgg gcgcgcggct gtttctgcac 180

tatggtgacc tgatcgacgg aacccggttg gtgaccctgc tgagcaccat cgaacccgac 240

gaggtgtaca acctggcggc gcagtcacac gtgcgggtga gcttcgacga acccgtgcac 300

accggtgaca ccaccggcat gggatccatg cgactgctgg aagccgttcg gctctctcgg 360

gtgcactgcc gcttctatca ggcgtcctcg tcggagatgt tcggcgcctc gccgccaccg 420

cagaacgagc tgacgccgtt ctacccgcgg tcaccgtatg gcgccgccaa ggtctattcg 480

tactgggcga cccgcaatta tcgcgaagcg tacggattgt tcgccgttaa cggcatcttg 540

ttcaatcacg aatcaccgcg gcgcggtgag acgttcgtga cccgaaagat caccagggcc 600

gtggcacgca tcaaggccgg tatccagtcc gaggtctata tgggcaatct ggatgcggtc 660

cgcgactggg ggtacgcgcc cgaatacgtc gaaggcatgt ggcggatgct gcagaccgac 720

gagcccgacg acttcgtttt ggcgaccggg cgcggtttca ccgtgcgtga gttcgcgcgg 780

gccgcgttcg agcatgccgg tttggactgg cagcagtacg tgaaattcga ccaacgctat 840

ctgcggccca ccgaggtgga ttcgctgatc ggcgacgcga ccaaggctgc cgaattgctg 900

ggctggaggg cttcggtgca cactgacgag ttggctcgga tcatggtcga cgcggacatg 960

gcggcgctgg agtgcgaagg caagccgtgg atcgacaagc cgatgatcgc cggccggaca 1020

tga 1023

<210> 2

<211> 340
<212> PRT
<213> mycobacterium tuberculosis

<400> 2

Met Lys Arg Ala Leu Ile Thr Gly Ile Thr Gly Gln Asp Gly Ser Tyr
1 5 10 15

Leu Ala Glu Leu Leu Leu Ala Lys Gly Tyr Glu Val His Gly Leu Ile
20 25 30

Arg Arg Ala Ser Thr Phe Asn Thr Ser Arg Ile Asp His Leu Tyr Val
35 40 45

Asp Pro His Gln Pro Gly Ala Arg Leu Phe Leu His Tyr Gly Asp Leu
50 55 60

Ile Asp Gly Thr Arg Leu Val Thr Leu Leu Ser Thr Ile Glu Pro Asp
65 70 75 80

Glu Val Tyr Asn Leu Ala Ala Gln Ser His Val Arg Val Ser Phe Asp
85 90 95

Glu Pro Val His Thr Gly Asp Thr Thr Gly Met Gly Ser Met Arg Leu
100 105 110

Leu Glu Ala Val Arg Leu Ser Arg Val His Cys Arg Phe Tyr Gln Ala
115 120 125

Ser Ser Ser Glu Met Phe Gly Ala Ser Pro Pro Pro Gln Asn Glu Leu
130 135 140

Thr Pro Phe Tyr Pro Arg Ser Pro Tyr Gly Ala Ala Lys Val Tyr Ser
145 150 155 160

Tyr Trp Ala Thr Arg Asn Tyr Arg Glu Ala Tyr Gly Leu Phe Ala Val
165 170 175

Asn Gly Ile Leu Phe Asn His Glu Ser Pro Arg Arg Gly Glu Thr Phe
180 185 190

Val Thr Arg Lys Ile Thr Arg Ala Val Ala Arg Ile Lys Ala Gly Ile
195 200 205

Gln Ser Glu Val Tyr Met Gly Asn Leu Asp Ala Val Arg Asp Trp Gly

210 215 220

Tyr Ala Pro Glu Tyr Val Glu Gly Met Trp Arg Met Leu Gln Thr Asp
225 230 235 240

Glu Pro Asp Asp Phe Val Leu Ala Thr Gly Arg Gly Phe Thr Val Arg
245 250 255

Glu Phe Ala Arg Ala Ala Phe Glu His Ala Gly Leu Asp Trp Gln Gln
260 265 270

Tyr Val Lys Phe Asp Gln Arg Tyr Leu Arg Pro Thr Glu Val Asp Ser
275 280 285

Leu Ile Gly Asp Ala Thr Lys Ala Ala Glu Leu Leu Gly Trp Arg Ala
290 295 300

Ser Val His Thr Asp Glu Leu Ala Arg Ile Met Val Asp Ala Asp Met
305 310 315 320

Ala Ala Leu Glu Cys Glu Gly Lys Pro Trp Ile Asp Lys Pro Met Ile
325 330 335

Ala Gly Arg Thr
340

<210> 3
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 3

Val Lys Arg Ala Leu Ile Thr Gly Ile
1 5

<210> 4
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 4

Ala Leu Ile Thr Gly Ile Thr Gly Gln
1 5

<210> 5
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 5

Gly Ile Thr Gly Gln Asp Gly Ser Tyr Leu
1 5 10

<210> 6
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 6

Gly Gln Asp Gly Ser Tyr Leu Ala Glu Leu
1 5 10

<210> 7
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 7

Gln Asp Gly Ser Tyr Leu Ala Glu Leu
1 5

<210> 8
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 8

Leu Leu Leu Ala Lys Gly Tyr Glu Val
1 5

<210> 9
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 9

Leu Ala Lys Gly Tyr Glu Val His Gly Leu
1 5 10

<210> 10
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 10

Ala Lys Gly Tyr Glu Val His Gly Leu
1 5

<210> 11
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 11

Lys Gly Tyr Glu Val His Gly Leu
1 5

<210> 12
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 12

Ala Ser Thr Phe Asn Thr Ser Arg Ile
1 5

<210> 13
<211> 10

<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 13

Thr Phe Asn Thr Ser Arg Ile Asp His Leu
1 5 10

<210> 14
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 14

Phe Asn Thr Ser Arg Ile Asp His Leu
1 5

<210> 15
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 15

Pro His Gln Pro Gly Ala Arg Leu Phe
1 5

<210> 16
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 16

His Gln Pro Gly Ala Arg Leu Phe Leu
1 5

<210> 17
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 17

Gln Pro Gly Ala Arg Leu Phe Leu
1 5

<210> 18
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 18

Arg Leu Phe Leu His Tyr Gly Asp Leu
1 5

<210> 19
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 19

Leu Phe Leu His Tyr Gly Asp Leu
1 5

<210> 20
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 20

Thr Arg Leu Val Thr Leu Leu Ser Thr Ile
1 5 10

<210> 21
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 21

Arg Leu Val Thr Leu Leu Ser Thr Ile
1 5

<210> 22
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 22

Leu Leu Ser Thr Ile Glu Pro Asp Glu Val
1 5 10

<210> 23
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 23

Ala Ser Pro Pro Pro Gln Asn Glu Leu
1 5

<210> 24
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 24

Phe Tyr Pro Arg Ser Pro Tyr Gly Ala Ala
1 5 10

<210> 25
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 25

Tyr Pro Arg Ser Pro Tyr Gly Ala Ala

1 5

<210> 26
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 26

Pro Arg Ser Pro Tyr Gly Ala Ala Lys
1 5

<210> 27
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 27

Ser Tyr Trp Ala Thr Arg Asn Tyr Arg
1 5

<210> 28
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 28

Asn Tyr Arg Glu Ala Tyr Gly Leu
1 5

<210> 29
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 29

Thr Phe Val Thr Arg Lys Ile Thr Arg Ala
1 5 10

<210> 30
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 30

Ala Val Ala Arg Ile Lys Ala Gly Ile
1 5

<210> 31
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 31

Val Ala Arg Ile Lys Ala Gly Ile
1 5

<210> 32
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 32

Arg Ile Lys Ala Gly Ile Gln Ser Glu Val
1 5 10

<210> 33
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 33

Ile Lys Ala Gly Ile Gln Ser Glu Val
1 5

<210> 34
<211> 9
<212> PRT

<213> artificial sequence

<220>
<223> Peptide

<400> 34

Gln Ser Glu Val Tyr Met Gly Asn Leu
1 5

<210> 35
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 35

Val Tyr Met Gly Asn Leu Asp Ala Val
1 5

<210> 36
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 36

Tyr Met Gly Asn Leu Asp Ala Val Arg
1 5

<210> 37
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 37

Trp Gly Tyr Ala Pro Glu Tyr Val
1 5

<210> 38
<211> 9
<212> PRT
<213> artificial sequence

<220>

<223> Peptide

<400> 38

Tyr Ala Pro Glu Tyr Val Glu Gly Met
1 5

<210> 39
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 39

Glu Pro Asp Asp Phe Val Leu Ala Thr
1 5

<210> 40
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 40

Val Leu Ala Thr Gly Arg Gly Phe Thr Val
1 5 10

<210> 41
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 41

Leu Ala Thr Gly Arg Gly Phe Thr Val
1 5

<210> 42
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 42

Gln Tyr Val Lys Phe Asp Gln Arg Tyr Leu
1 5 10

<210> 43
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 43

Ser Leu Ile Gly Asp Ala Thr Lys Ala
1 5

<210> 44
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 44

Gly Asp Ala Thr Lys Ala Ala Glu Leu
1 5

<210> 45
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 45

Asp Ala Thr Lys Ala Ala Glu Leu Leu
1 5

<210> 46
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 46

Leu Leu Gly Trp Arg Ala Ser Val His
1 5

<210> 47
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 47

Arg Ala Ser Val His Thr Asp Glu Leu
1 5

<210> 48
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> Peptide

<400> 48

Ser Val His Thr Asp Glu Leu Ala Arg
1 5

<210> 49
<211> 24
<212> DNA
<213> artificial sequence

<220>
<223> Primer

<400> 49
agatctgtga agcgagcgct catc 24

<210> 50
<211> 21
<212> DNA
<213> artificial sequence

<220>
<223> Primer

<400> 50
agatcttgtc cggccggcga t 21

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/09345

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/04 C07K14/35 A61P31/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 23624 A (DORAN TIM ;MILLAR DOUGLAS (AU); FORD JOHN (GB); LOUGHLIN MARK (GB)) 3 July 1997 (1997-07-03) Seq ID nos 9-12 and 32, 33: seq ID no 33 has 100% identity over the whole length with the amino acid sequence ID no 2 page 27, line 5 -page 35, line 23 page 20, line 8 -page 20, line 18 ---	1-36
X	WO 99 54487 A (BILLAULT ALAIN ;COLE STEWART (FR); GORDON STEPHEN (FR); BUCHRIESER) 28 October 1999 (1999-10-28) Seq ID 1: 100% identity with seq ID no 1 of the application --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 December 2002	20/12/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Teyssier, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	MATTOW JENS ET AL: "Identification of proteins from Mycobacterium tuberculosis missing in attenuated Mycobacterium bovis BCG strains." ELECTROPHORESIS, vol. 22, no. 14, August 2001 (2001-08), pages 2936-2946, XP002223957 ISSN: 0173-0835 the whole document	1-36
A	COLE S T ET AL: "Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence" NATURE, vol. 393, 11 June 1998 (1998-06-11), pages 537-544, XP002087941 ISSN: 0028-0836	
A	BEHR M A ET AL: "Comparative genomics of BCG vaccines by whole-genome DNA microarray" SCIENCE, vol. 284, no. 5419, 28 May 1999 (1999-05-28), pages 1520-1523, XP002200574 ISSN: 0036-8075	
A	STOVER C K ET AL: "NEW USE OF BCG FOR RECOMBINANT VACCINES" NATURE, vol. 351, no. 6326, 6 June 1991 (1991-06-06), pages 456-460, XP000605495 ISSN: 0028-0836	
A	LOWRIE D B ET AL: "TOWARDS A DNA VACCINE AGAINST TUBERCULOSIS" VACCINE, vol. 12, no. 16, 1994, pages 1537-1540, XP002026338 ISSN: 0264-410X	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/09345

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9723624 A	03-07-1997	AU 735112 B2	28-06-2001
		AU 1202797 A	17-07-1997
		EP 0870032 A2	14-10-1998
		WO 9723624 A2	03-07-1997
		NZ 502423 A	30-11-2001
		US 6156322 A	05-12-2000
WO 9954487 A	28-10-1999	US 6183957 B1	06-02-2001
		AU 3164099 A	08-11-1999
		EP 1071797 A2	31-01-2001
		WO 9954487 A2	28-10-1999